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Jordan Journal of Pharmaceutical Sciences

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***Jordan Journal of Pharmaceutical Sciences* (*JJPS*)** is a bimonthly open-access peer reviewed journal funded by the Scientific Research Fund at Ministry of Higher Education and Research and hosted by the Deanship of Research at the University of Jordan. JJPS is dedicated to various disciplines of pharmaceutical and allied sciences. JJPS publishes manuscripts on original work, either experimental or theoretical in the following areas:

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INTRODUCTION

The Jordan Journal of Pharmaceutical Sciences (**JJPS**) is a peer-reviewed Journal, which publishes original research work that contributes significantly to further the scientific knowledge in pharmaceutical sciences' fields including pharmaceutical/medicinal chemistry, drug design and microbiology, biotechnology and industrial pharmacy, instrumental analysis, phytochemistry, biopharmaceutics and Pharmacokinetics, clinical pharmacy and pharmaceutical care, pharmacogenomics, bioinformatics, and also **JJPS** is welcoming submissions in pharmaceutical business domain such as pharmacoeconomics, pharmaceutical marketing, and management. Intellectual property rights for pharmaceuticals, regulations and legislations are also interesting topics welcomed from our colleagues in Schools of Law.

On a current topic in Pharmaceutical Sciences are also considered for publication by the Journal. **JJPS** is indexed in SCOPUS (Q3). It's a journal that publishes 4 issues per year since 2021 in (**March, June, September, December**). The Editorial Team wishes to thank all colleagues who have submitted their work to JJPS). If you have any comments or constructive criticism, please do not hesitate to contact us at jjps@ju.edu.jo. We hope that your comments will help us to constantly develop **JJPS** as it would be appealing to all our readers.

Prof Ibrahim Alabbadi
Editor-in-Chief
School of Pharmacy- The University of Jordan
Amman 11942- Jordan

Volume 17, 2024

Letter from the Editor-in-Chief

Typically, Food and Drug Administration (FDA) organizations or health authorities in countries worldwide perform all necessary investigations before granting approval for any medication or any type of food suitable for human consumption. But what happens when these authorities do not exist? Can we expect peace in 2024? We all hope every human, irrespective of their geographical location, can live in peace and enjoy good health, with all essential life necessities readily available. The World Health Organization's recent definition of health refers to a state of complete physical, social, and mental well-being, NOT ONLY the absence of disease or infirmity. Health-Related Quality of Life is a fundamental right for any human living on earth. But what if there is no food or medication, or if these essentials are even prohibited?

The Jordan Journal of Pharmaceutical Sciences (JJPS) team includes numerous scholar colleagues from universities in Gaza which have been demolished. These colleagues may still be alive or sadly, they may have already lost their lives. Numerous professors supported the JJPS by acting as peer reviewers and submitting quality scientific articles under incredibly challenging circumstances. We tip our hats to all of them and hope that God, with his immediate power, will grant life, peace, and security as Ramadan commences in March.

The JJPS editorial board has already initiated the second phase of a three-year term, following renewal approval from the Jordanian Ministry of Higher Education, which underscores our tremendous teamwork and significant progress. The JJPS' scores in international scientific databases, such as SCOPUS, continue to improve – our Q3 score is now close to Q2. Additionally, we've seen a continued influx of submissions from increasingly diverse countries, including places in North Africa, Europe, the USA, Canada, Australia, and Southeast Asia. We've also noticed a significant reduction in time from submission through revision to the decision-making process, and with the rise in ambiguity due to AI and ChatGPT programs, the need for similarity report checks has become essential.

Best regards

Prof Ibrahim Alabbadi
Editor-in-Chief

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Recent Advances in Development of Vesicular Carrier for Transdermal Drug Delivery: A Review

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ABSTRACT

Transdermal drug delivery has gained significant attention as a non-invasive and convenient method for administering drugs. However, the stratum corneum, the outermost layer of the skin, poses a significant barrier to drug permeation. To overcome this challenge, vesicular carriers have emerged as promising systems for enhancing drug delivery through the skin. This review highlights recent advances in the development of vesicular carriers for transdermal drug delivery. Liposomes, niosomes, transfersomes, ethosomes, and solid lipid nanoparticles are among the commonly used vesicular carriers. These carriers offer advantages such as improved drug solubility, prolonged drug release, and enhanced drug stability. Additionally, they can encapsulate a wide range of drugs, including hydrophilic and lipophilic compounds. Various strategies have been employed to optimize vesicular carriers for transdermal drug delivery. These include modifying the vesicle composition, size, and surface charge to enhance skin penetration. The incorporation of penetration enhancers, such as surfactants, has also been explored to improve drug permeation across the skin. Furthermore, advancements in nanotechnology have led to the development of novel vesicular carriers, such as nanostructured lipid carriers and elastic liposomes. These carriers offer improved drug loading capacity, sustained release profiles, and enhanced skin penetration. Moreover, the use of vesicular carriers has shown promise in delivering a wide range of therapeutic agents, including small molecules, peptides, proteins, and genetic material. The ability to encapsulate and deliver these diverse drug entities opens new possibilities for transdermal drug delivery in various therapeutic areas.

Keywords: Transfersomes, liposomes, niosomes, ethosomes, ufasomes, sphingosomes and cubosomes, transdermal drug delivery, vesicular formulation.

I. INTRODUCTION

From 2021 to 2030, the global transdermal drug delivery systems market is expected to increase at a compound annual growth rate (CAGR) of 4.9 percent, rising from $52,476.50 million in 2020 to $87,322.40 million in 2030[(1)]. Transdermal drug delivery systems, which transport medications through the skin for therapeutic purposes, serve as an alternative to oral, intravascular, subcutaneous, and transmucosal routes. Transdermal drug delivery systems provide a painless, systematic form of medication delivery by applying the medicine to healthy, unbroken skin [(1-2)].

The skin serves as a popular site for drug administration for both local and systemic effects since it is the largest organ of the body and provides a direct entrance for medication into the systemic circulation. This bypasses all of the issues associated with the oral and parenteral routes. However, due to its nature as a barrier, the skin presents a significant obstacle to drug penetration, reducing transdermal bioavailability. Several approaches have been employed to improve drug penetration through the skin, and ongoing research has led to the development of newer vehicles/carriers, specifically vesicular carriers, lipid-

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microemulsions, and other systems [2].

Furthermore, the development of newer technologies for delivering drug molecules along with safe penetration enhancers and the utilization of vesicular carriers have reignited interest in constructing transdermal drug delivery systems for medications previously deemed unsuitable [3].

Drug delivery from vesicle carriers, such as liposomes and niosomes, in transdermal formulations has been studied for a variety of purposes. However, both are inherently unstable, exhibit poor skin permeation, and lower entrapment efficiency. In addition, they increase Trans Epidermal Water Loss (TEWL), so they are mostly used in topical delivery in limited amounts [4].

According to recent studies, traditional vehicles such as suspensions and emulsions and traditional carriers such as liposomes and niosomes, can cause potential damage to the stratum corneum and reduce its thickness. They can also lead to skin dryness, demonstrate low penetration through the stratum corneum, and exhibit low entrapment efficiency [5]. However, some of these issues can be overcome by designing a unique vehicle or vesicular carrier.

The stratum corneum (SC), viable epidermis, dermis, and the subcutaneous tissue constitute the four primary layers of human skin. They are, on average, 0.5 mm thick (varying from 0.05 mm to 2 mm). The SC is a thick (10–20 µm) hydrophobic surface layer that contains 10–15 layers of interdigitated corneocytes that are regularly shed and replaced. Extracellular lipid constitutes 10% of the dry weight of this layer, while intracellular protein accounts for the remaining 90% (mainly keratin). As the cells differentiate during their migration to the surface, the phospholipid content decreases, and the sphingolipid (glucosylceramide and ceramide) and cholesterol content simultaneously increases. The SC is devoid of phospholipids but is enriched with ceramides and neutral lipids (cholesterol, fatty acids, cholesteryl esters). The skin's barrier lipids are tightly regulated, and any damage to them prompts active synthetic processes to replenish them [4, 6].

The relative humidity of the surroundings significantly impacts the skin's barrier function. When transitioning from a humid to a dry environment, transepidermal water loss can increase by 6–7 times. The reduction of lamellar bodies in the outermost stratum granulosum, the deposition of lamellar body contents at the stratum granulosum-stratum corneum interface, and the decrease in the amount of intercellular lamellae in the stratum corneum all could affect the barrier function. Conversely, transitioning from a wet environment to a dry one quickly stimulates epidermal growth, and vice versa [6, 7, 8].

The stratum corneum is a complex tissue due to the presence of multiple self-regulating enzymatic systems. It is metabolically active and undergoes dynamic structural alterations. This tissue encompasses numerous defensive (protective) functions, each with its structural and metabolic foundation. Newer metabolically-based methods have shown promise in broadening the range of drugs that can be delivered transdermally in hairless mouse epidermis when used alone or in conjunction with traditional methods. While these new approaches are highly promising, they may raise concerns about the risks associated with a significantly permeabilized stratum corneum, should they prove equally effective in human skin [6][8].

Instruments like the Vapometer, which detects transepidermal water loss, are utilized to determine the permeability barrier function (TEWL). The accuracy of TEWL measures has been verified both in vivo and ex-vivo using human and rodent models [9,10].

When salicylic acid (SA) penetration was evaluated in barrier-perturbed skin compared to unmodified skin in the same individual, the average increase was 2.2-fold in acetone-treated skin, 46-fold in moderate dermatitis, and 146- and 157-fold in severe dermatitis and tape-stripped skin, respectively. SA penetration was shown to be highly correlated with TEWL measurements of barrier disruption [7].

Hydration and chemical enhancers can also be used to modify the stratum corneum, or it can be bypassed/

eliminated via microneedles, ablation, and follicular administration. Examples of electrically assisted procedures include ultrasound, iontophoresis, electroporation, magnetophoresis, and photomechanical waves. The interaction of chemical enhancers, ultrasound, iontophoresis, and electroporation is particularly fascinating[11].

A novel high throughput (HTP) method for formulation screening is proposed, which is at least 50-fold more efficient in terms of skin utilization and up to 30-fold more efficient in terms of hold-up times than current methods (Franz diffusion cells). It is based on the conductivity of the skin and the penetration of mannitol into the skin. This strategy was used to conduct at least 100 tests in a single day [12].

This article provides a detailed account of various vesicular carriers, their formulation characteristics, pros and cons, along with a compilation of recent trends in transdermal drug delivery technology with respect to vesicular carriers.

II. VESICULAR CARRIERS

Mezei and Gulasekharam were the first to demonstrate that liposomes could be useful for topical therapy in 1980(13). Vesicles are known as water-filled colloidal particles. The walls of these capsules are made up of bilayers of amphiphilic molecules. In the presence of excess water, these amphiphilic compounds can form either one (unilamellar vesicles) or multiple (multilamellar vesicles) concentric bilayers [14]. Hydrophilic drugs can be enclosed in the internal aqueous compartment, whereas the vesicle bilayer can bind amphiphilic, lipophilic, and charged hydrophilic drugs through hydrophobic and/or electrostatic interactions [15].

The vesicles are predominantly composed of phospholipids or non-ionic surfactants [15, 16]. These two types of vesicles are referred to as liposomes and niosomes. The size, charge, thermodynamic phase, lamellarity, and bilayer elasticity of vesicles are all influenced by the composition of the vesicles. These physicochemical properties significantly impact vesicle behavior and, consequently, their effectiveness as a drug delivery method. They can be classified into the following roles:

1. Serve as drug carriers to transfer entrapped drug molecules into or across the skin.
2. Act as penetration enhancers by allowing individual lipid components to penetrate the stratum corneum, leading to alterations in the intercellular lipid lamellae within this skin layer.
3. Act as a depot for the sustained release of dermally active compounds over time.
4. Provide a regulated transdermal delivery method by acting as a rate-limiting membrane barrier for modulating systemic absorption.

In vitro permeation studies have demonstrated that liquid-state vesicles are more successful at increasing drug transport than gel-state vesicles [17, 18, 19, 20]. In vivo confirmation of these findings has been recently published [21].

1. Conventional Liposomes: The first generation of liposomes is termed conventional liposomes. They are a type of vesicle composed of a lipid bilayer that surrounds aqueous compartments and can be composed of cationic, anionic, or neutral (phospholipid) lipids, as well as cholesterol. Natural phospholipids or lipids, such as 1,2-distearyl-sn-glycerin-3-phosphatidylcholine (DSPC), sphingomyelin, lecithin, and monosialoganglioside, have been used in traditional liposome formulations. They have been widely used to transport hydrophilic and lipophilic compounds [22, 23, 24, 25, 26].

Table 1: Methods of separating carriers for transdermal drug delivery

S.No.	Carriers	Method Name	Reference
1	**Liposomes**	• Thin-film hydration process • Reverse-phase evaporation process • Solvent injection process	(27)
2	**Transfersomes**	• Thin film hydration technique/rotary evaporation-sonication method • Vortexing-sonication method • Modified handshaking process • Suspension homogenization process • Reverse-phase evaporation method • High-pressure homogenization technique	(28)
3	**Ethosomes**	• Cold method • Hot method • Mechanical dispersion method	(29)
4	**Niosomes**	• Ether injection method • Sonication • Multiple membrane extrusion method • Reverse phase evaporation technique (REV) • G.Trans membrane pH gradient (inside acidic) drug uptake process	(30)
5	**Ufasomes**	• Thin film hydration method • By addition of alcohol • Autopoeticprocess	(31)
6	**Sphingosomes**	• Lipid flimformation (hand shaking method) • Solvent spherule method • Calcium induced fusion method	(32)
7	**Cubosomes**	• Top-down approach • Bottom-up approach	(33)

Table 2: Carriers composition

Carriers	Class	Example	Use	Reference
Liposomes	Phospholipids	Soya phosphatidyl choline, egg phosphatidylcholine, dipalmitoylphosphatidyl choline	Vesicles forming component	**(8, 23, 24, 25)**
	Polyglycol	Propylene glycol, Transcutol RTM	Skin penetration enhancer	
	Cholesterol	Cholesterol	Provides stability to the vesicle membrane	
Transfersomes	Phospholipids	Soya phosphatidyl choline, egg phosphatidylcholine, dipalmitoylphosphatidyl choline	Vesicles forming component	**(34, 35)**
	Surfactants	Sodium cholate, Sodium deoxycholate, Tween-80, Span-80, Tween 20	Vesicles forming component	
	Solvents	Ethanol, methanol, isopropyl alcohol, chloroform	As Solvents	
	Buffering agents	Saline phosphate buffer (pH 6.4), phosphate buffer pH 7.4	As hydrating medium	
Ethosomes	Phospholipids	Soya phosphatidyl choline, egg phosphatidylcholine, dipalmitoylphosphatidyl choline	Vesicles forming component	(36, 37, 38, 39, 40, 41)
	Polyglycol	Propylene glycol, Transcutol RTM	Skin penetration enhancer	
	Alcohol	Ethanol, isopropyl alcohol	Provides softness to the vesicle membrane, as a penetration enhancer	
	Cholesterol	Cholesterol	Provides stability to the vesicle membrane	
	Dyes	Rhodamine -123, Rhodamine red, Fluorescence isothiocyanate,	For characterization study	
	Vehicle	Carbopol 934	As a gel provider	
Niosomes	Nonionic surfactants	Alkyl glycerol, alkyl glycosides, polysorbate 60,	Vesicles forming component	(42, 43, 44, 45)
	Cholesterol	Cholesterol	Provides stability	
	Charge inducer	diacetyl phosphate (DCP) and phosphotidic acid.	Increases stability	
	Hydrating medium	phosphate buffer	Buffering agent	
Ufasomes	Fatty acids	10% oleic and linoliec acid	Vesicle forming component	(46, 47, 48)
	Solvents	Chloroform, stream of nitrogen	membrane permeability	
	Buffering agent	Tris-hydroxymethyl aminomethane buffer (pH 8-9)	Hydrating medium	
Sphingosomes	Sphingolipids	Egg, brain, milk, soybean, plant yeast	Vesicle forming component	(49, 50)
	Cholesterol	Cholesterol	membrane stability	
Cubosomes	Amphiphilic lipids	glyceryl monooleate	Vesicle forming component	(51, 52, 53)
	Stabilizers	Poloxamer 407, polyethylene glycol 400	Membrane stability	

Table 3: Advantages and disadvantages of liposomes[54, 55]

Advantages	Disadvantages
Liposomes have the ability to form complexes with both negatively and positively charged substances.	The expense of production is high.
Liposomes provide some protection for DNA from degradative processes.	Encapsulated drug/molecule leakage and fusion
Liposomes have the ability to carry huge amounts of DNA, maybe as large as a chromosome.	Oftentimes, phospholipids are subjected to oxidation and hydrolysis-like processes.
Liposomes have the ability to target specific cells or regions.	Short half-life
Effects of improved pharmacokinetics	Low solubility
Increase in the drug's effectiveness and therapeutic index	Fewer stables

The key goals of a method for liposome nanoformulation formation are the generation of monodispersed particles with the desired degree of lamellarity, efficient drug inclusion, and long-term colloidal stability of products [13, 27, 55]. The primary processes involved in traditional liposome preparation methods include:

1. Dissolution of lipids in an organic solvent;
2. Drying down the resultant lipid solution to remove the organic solvent;
3. Hydrating the lipid with an aqueous media (followed by agitation/stirring);
4. Downsizing (and/or changing the lamellarity);
5. Post-formation processing (purification, sterilization)[56].

Mechanism of Action of Liposomes:

A liposome consists of a region of aqueous solution inside a hydrophobic membrane. Hydrophobic chemicals can easily dissolve into the lipid membranes. In this way, liposomes can carry both hydrophilic and hydrophobic molecules, although the extent of the location of the drug will depend on its physicochemical characteristics and the composition of the lipid. For the delivery of necessary drug molecules to the site of action, the lipid bilayers can fuse with other bilayers of the cell (cell membrane), which would release the liposomal contents.

Adsorption

(Interaction of liposomes within cell membrane)

↓

Endocytosis

(Cell surface membrane is engulfed and internalised into the liposomes)

↓

Fusion

(Liposome lipid bilayers fuse with the lipoidal cell membrane, resulting in direct delivery of liposomal contents into the cytoplasm.)

↓

Lipid exchange

(Lipid transfer proteins in the cell, recognizes liposomes and cause lipid exchange)

For instance, cancer cells consume vast amounts of fats to meet their rapid growth requirements, and they perceive liposomes (laden with anti-cancer drugs) as a potential source of nutrients. When targeted by liposomes, they are absorbed. Once the anti-cancer medications are released from the liposome into the tumor site, they destroy the cancer cells [57, 58].

Transfersomes: Transfersomes are a specific type of liposome composed of phosphatidylcholine and an edge activator (e.g., sodium cholate, sodium deoxycholate, span 80, and Tween 80). They are soft, malleable vesicles designed to deliver active substances more effectively [59]. The name is derived from the Latin word 'transfere,' meaning 'to carry across,' and the Greek word 'soma,' meaning 'body.' The pros and cons of transfersomes are outlined below (Table 4) [60, 61, 62, 63].

Table 4: Advantages and disadvantages of transfersomes [64]

Advantages	Disadvantages
A constant infusion of a substance is delivered via transdermal medication over a long period of time.	Drugs having hydrophilic structures pass through the skin too slowly to be of therapeutic value.
These systems allow for self-administration.	Because the patch size limits the amount that can be administered, the drug molecule must be powerful.
They are appropriate for medications having a limited therapeutic window.	High medication doses are not recommended.
Dosing frequency is reduced due to a longer duration of effect.	It is possible that skin irritation and hypersensitivity reactions will occur.
Bioavailability has improved.	It is not possible to deliver drugs that require high blood levels.
Improved therapy and fewer side effects	Because to oxidative breakdown, it is chemically unstable.

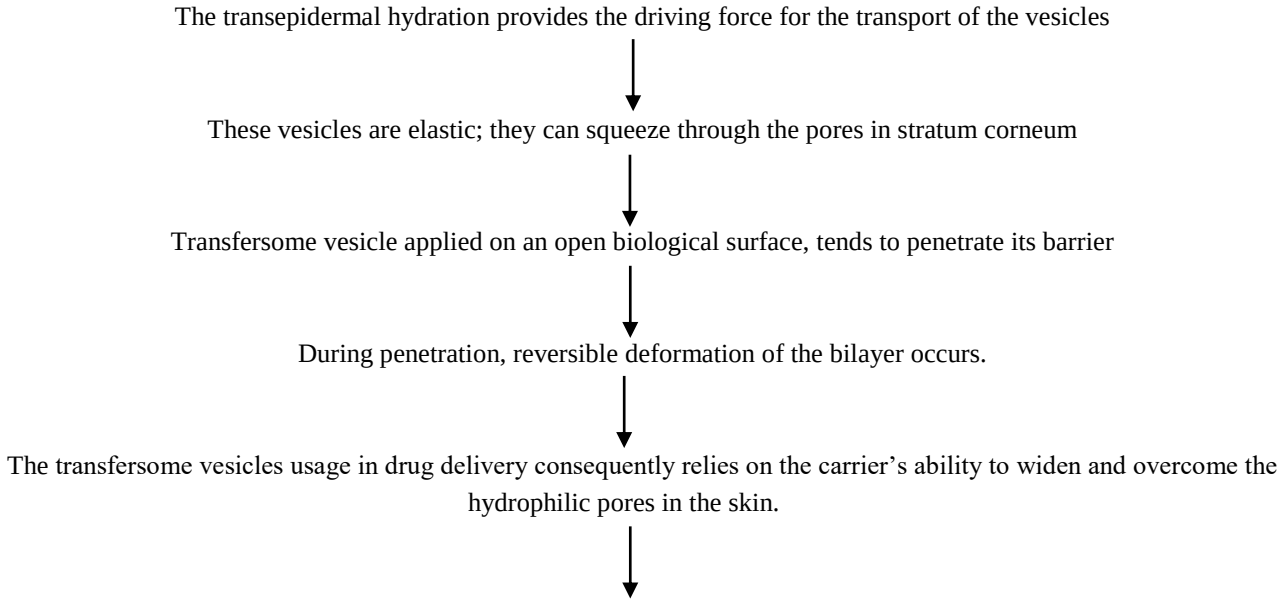
2.1 Mechanism of penetration of transfersomes

Transferosomes overcome the skin penetration difficulty Transferosomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of the stratum corneum. Two mechanisms of action have been proposed.

1. Transferosomes act as drug vectors, remaining intact after penetrating the skin.

2. Transferosomes act as penetration enhancers, disrupting the highly organized intercellular lipids from the stratum corneum, thereby facilitating drug molecule penetration into and across the stratum corneum. (Patel R., Singh S.K., Singh. S, Sheth N.R., Gendle R. "Development and Characterization of Curcumin Loaded Transferosome for Transdermal Delivery" Journal of Pharmaceutical Research and Science, 2009; 1(4): 71-80).

The formation of an "osmotic gradient" due to the evaporation of water on the skin surface is the mechanism for penetration [65].



3. Ethosomes: Ethosomes are soft, flexible lipid vesicles primarily composed of phospholipids, a high concentration of alcohol (20-45%), and water. Touitou and her colleagues initially developed ethosomes in 1997 [66, 67]. Owing to their high deformability, these carriers possess fascinating properties connected to their capacity to fully permeate human skin. The physicochemical properties of ethosomes allow these vesicular phospholipids to function as the vesicle-forming component of the ethosomal system. Phospholipids with various chemical structures such as phosphatidyl choline and phosphatidyl ethanolamine are utilized at concentrations ranging from 0.5 to 10%.

Table 5: Advantages and disadvantages of ethosomes

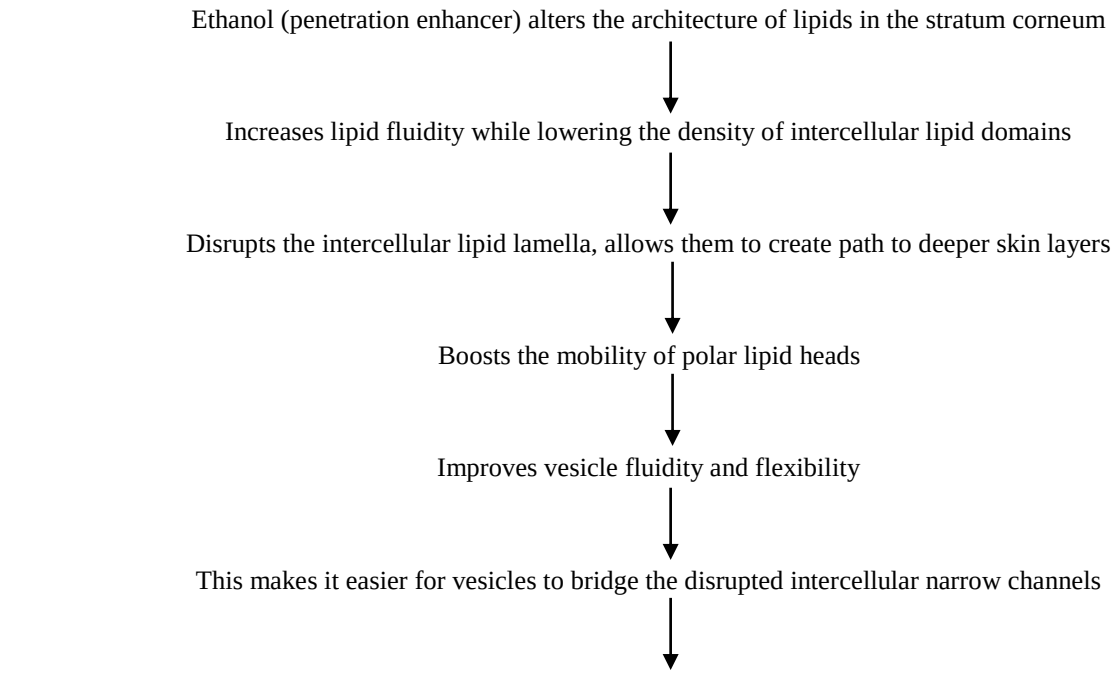
Advantages	Disadvantages
In its formulation, it uses non-toxic raw materials.	Poorly shelled ethosomes may clump together, resulting in precipitation.
Large molecule delivery.	Excipients and enhancers in drug delivery systems cause skin irritation or dermatitis.
Drug permeability through the skin is improved for transdermal drug delivery.	The loss of product occurs when ethosomes are transferred from the organic to the aqueous layer.
Ethosomes have the highest transdermal flux, which improves drug diffusion through deeper layers of skin.	The drug's molecular size should be small enough to be absorbed via the skin.
Under both occlusive and non-occlusive situations, ethosomes improve skin delivery.	The practical yield is poor.

3.1 Mechanism of skin penetration

The main advantage of ethosomes over liposomes is the increased permeation of the drug. The mechanism of drug absorption from ethosomes is not fully understood, but it likely occurs in two phases:

1. Ethanol Effect: Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration-enhancing effect is well established. Ethanol penetrates into intercellular lipids, increases the fluidity of cell membrane lipids, and decreases the density of the lipid multilayer of the cell membrane.
2. Ethosome Effect: The increased cell membrane lipid fluidity caused by the ethanol in ethosomes results in increased skin permeability. As a result, the ethosomes permeate very easily into the deep skin layers, where they fuse with skin lipids and release the drugs into the deeper layer of the skin.

The mechanism is illustrated below [68, 69].



4. Niosomes: Niosomes are a unique drug delivery technology that encapsulates drugs in a vesicle. The term niosome derives from the formation of the vesicle, which is composed of a bilayer of non-ionic surface-active chemicals. Niosomes are small, microscopic particles. Their size, on the nanometric scale, is misleading. Although niosomes are physically similar to liposomes, they have a few advantages. Recently, niosomes have been demonstrated to improve transdermal drug delivery and can also be employed in targeted drug delivery. Therefore, further research into these structures could lead to new drug delivery systems [70].

Niosomes are non-ionic surfactant-based vesicles made by hydrating synthetic nonionic surfactants, which can include cholesterol or other lipids. They are vesicular systems, similar to liposomes, that can transport both amphiphilic and lipophilic drugs. Since they are non-ionic, niosomes are a promising delivery route for drugs. By localizing the drug's effect to target cells, they are less hazardous and enhance the therapeutic index. The pros and cons of niosomes are outlined below [71].

Table 6: Advantages and disadvantages of niosomes

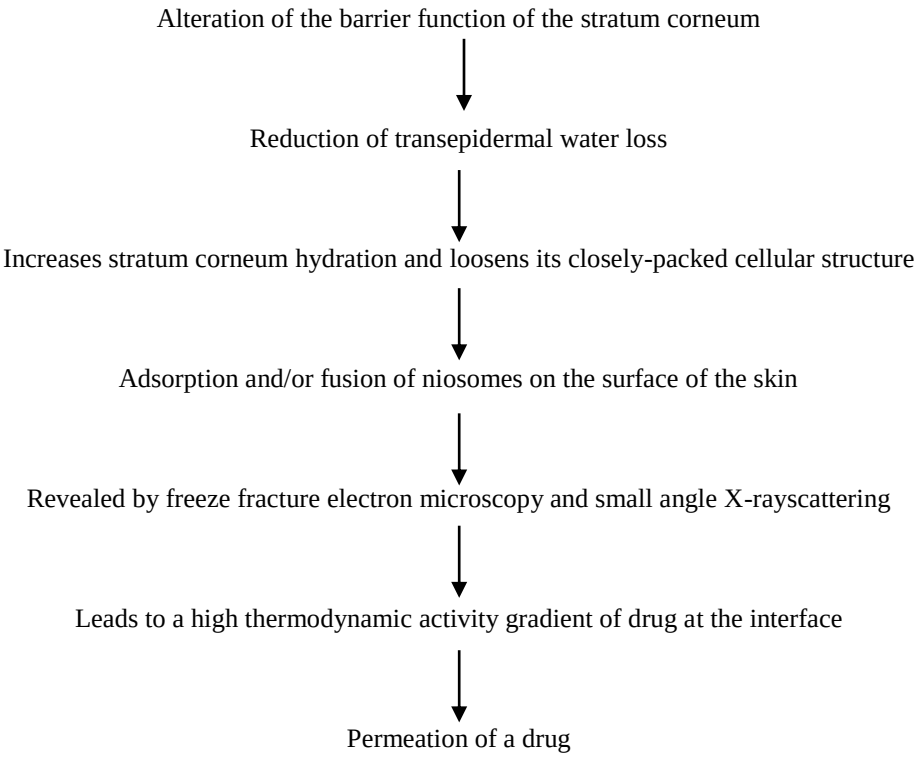
Advantages	Disadvantages
They are both osmotically active and osmotically stable.	Instability of the physical body
They improve the entrapped drug's stability.	Aggregation
Drug penetration through the skin can be improved.	Fusion
The surfactants are non-immunogenic, biodegradable, and biocompatible.	Entrapped drug leakage
Improve the drug's therapeutic performance by shielding it from the biological environment and limiting its effects to target cells, therefore lowering the drug's clearance.	Encapsulated medicines are hydrolyzed, reducing the shelf life of the dispersion[5-8]

4.1 Mechanism of skin penetration

Several mechanisms have been proposed to explain the ability of niosomes in transdermal and dermal drug delivery:

i) Niosomes diffuse from the stratum corneum layer of the skin as a whole. ii) New smaller vesicles are formed in the skin (re-formation of niosome vesicles). iii) Niosomes interact with the stratum corneum through aggregation, fusion, and adhesion to the cell surface, which causes a high thermodynamic activity gradient of the drug at the vesicle-stratum corneum surface. This is the driving force for the penetration of lipophilic drugs across the stratum corneum. iv) Niosomes may modify the stratum corneum structure, making the intercellular lipid barrier of the stratum corneum looser and more permeable. v) The non-ionic surfactant itself, the composing ingredient of niosome, acts as a permeation enhancer and might partially contribute to the improvement of drug permeation from niosomes [72].

The type of surfactant plays a significant role in modifying permeation using niosome vehicles. Niosomes fabricated from polyoxyethylene stearyl ether that exist in the gel state did not enhance estradiol permeation, whereas those prepared from polyoxyethylene lauryl ether and polyoxyethylene oleyl ether, both existing as liquid crystalline vesicles, significantly improved transport [73, 74]. Several mechanisms have been proposed, including:



5. OTHER NOVEL VESICULAR CARRIERS

5.1 Ufasomes: Ufasomes form when an evaporated film is physically agitated in the presence of a buffer solution. They are vesicles made up of long-chain unsaturated fatty acids. Colloidal suspensions of fatty acids and their ionized forms are referred to as fatty acid vesicles. It is an efficient way to deliver medications to an infection site quickly, with reduced opioid toxicity and side effects. Ufasome is a novel method to improve opioid absorption through the skin. Unsaturated fatty acids like linoleic and oleic acids are used as natural permeation enhancers in the production of ufasomes. Surfactants are often used with fatty acids to improve skin flexibility and medication transport across the skin membrane. Ufasomes enhance drug retention qualities inside the cell membrane of skin cells for an extended period. Ufasomes are soapy suspensions of closed lipid bilayers primarily composed of fatty acids [31, 75]. They typically maintain a narrow pH range of 7 to 9.1 in nature.

5.1.1 Mechanism of skin penetration[47, 76]

Fatty acid vesicles lower the phase transition temperature of lipids in biological membranes

↓

Bilayer membrane has a fusogenic tendency

↓

The vesicle membrane combines with skin lipids, allowing the contents of the vesicle to be released

↓

Fatty acid vesicles will serve as an effective carrier for increasing drug penetration into the stratum corneum

↓

resulting in lower toxicity

5.2 Sphingosomes: Sphingosomes are concentric, bilayered vesicles with an aqueous core surrounded by a membranous lipid bilayer primarily made up of natural or synthesized sphingolipids. Sphingosomes are composed of sphingolipids and cholesterol, with an internal aqueous environment that has a lower pH than the surrounding environment [77, 78, 79]. Sphingosomes are the key targeted lipid vesicular drug delivery method. They are constructed from a membranous lipid bilayer that surrounds an aqueous area in which the drug can be contained. Sphingosomes overcome the drawbacks of liposomes and niosomes due to their great resistance to acid hydrolysis and enhanced drug retention capabilities. Sphingosomes can be administered into the body via several routes, including parenteral, inhalation, oral, and transdermal. Sphingolipids, which are predominantly composed of amide and ester linkages, make up sphingosomes [32, 80, 81].

Table 7: Advantages and disadvantages of sphingosomes [82]

Advantages	Disadvantages
Provide tumor tissue with selective passive targeting.	Sphingolipids are more expensive.
Increase the therapeutic index and efficacy [89]	Entrapment efficacy is low.
The encapsulated agent's toxicity is reduced.	
Encapsulation improves stability.	

5.2.1 Mechanism of skin penetration [80]

There are various ways in which small unilamellar sphingosomal vesicles (SUSVs) interact with cells. These are as follows: stable adsorption, endocytosis, fusion, and lipid transfer.

Stable adsorption: Stable adsorption represents the association of intact vesicles with the cell surface. This process is mediated by non-specific electrostatic, hydrophobic or other forces present at the vesicles or the cell surface.

Endocytosis: Endocytosis is the uptake of intact vesicles into endocytotic vesicles and presumably results in their delivery to the lysosomal apparatus.

Fusion: Fusion is the simple merging of the vesicle's

bilayer with the plasma membrane bilayer, with the release of vesicle content into the cytoplasmic space.

Lipid Transfer: This involves the transfer of individual lipid molecules between vesicles and the cell surface without the cell association of aqueous vesicle content.

Stable adsorption

Adsorption of intact vesicles to the cell surface

(Non-specific electrostatic, hydrophobic, or other forces are involved in this process)

↓

Endocytosis

Intact vesiclestaken up by endocytotic vesicles and sent to the lysosomal apparatus.

↓

Fusion

Fusion of the bilayers of vesicles and plasma membranes

↓

with components releasing vesicle content into the cytoplasmic space.

↓

Lipid exchange

lipid molecules are transferred between vesicles and the cell surface without the need for aqueous vesicle content to be associated with the cell.

5.3 Cubosomes: Cubosomes are unique, sub-micron, nano-structured particles that represent a bicontinuous cubic liquid crystalline phase [(83)]. Cubosomes are self-assembled liquid crystalline particles of certain surfactants with a specific water-microstructure ratio and a solid-like rheology [(83)]. Cubosomes have the same microstructure as the parent cubic phase, but they possess a larger specific surface area and exhibit smaller viscosity dispersions than the bulk cubic phase [(84, 85, 86)]. The viscosity of the bulk cubic phases is higher than that of cubosomal dispersions [(87)]. Cubosomes are typically created by dispersing bulk cubic phase with high energy, then stabilizing the colloidal phase with polymeric surfactants. Cubic phase liquid crystals can be used for the controlled release of selected water-oil soluble compounds [(87, 88)]. Cubosomes comprise lipids, surfactants, and polymer molecules that have both polar and non-polar components.

Table 8: Advantages and disadvantages of cubosomes [89]

Advantages	Disadvantages
Hydrophilic and hydrophobic drugs, as well as amphiphilic drugs can be encapsulated.	In preparation, there is a low drug loading efficiency and drug leakage.
Have sustained drug delivery capabilities	Its stability acts as a deterrent, restricting their application.
Have qualities of biocompatibility and bioadhesivity	Because of the high viscosity, large-scale production can be challenging.

Table 9: Recent studies (Last 10 years) done in carriers for transdermal drug delivery

Carriers	Drug	Key findings	Indication	Reference
Liposomes	Econazole	Releases the drug at local sites of infection, may be through the action of lipase. Reduction of drug dosage and skin irritation	Anti-fungal	[90]
	Melanin	Deliver increased amounts of drugs to the site of action.	Hair growth	[91]
	Tetracaine	Delivery of drugs into the deeper skin strata.	Anaesthetic	[92]
	Tretinoin	Enhancing skin permeation in dermal delivery using different hydrophilic penetration enhancers. Increased cutaneous accumulation	Psoriasis	[93]
	Gentamycin	Drug showed increased survival rate of animal model and increased therapeutic efficacy	Pneumonia	[94, 95, 96]
	Vincristine	Enhanced vincristine cell uptake, penetration and concentration in tissues and organs and involved in the mononuclear phagocyte system	Leukemia	[96]

Carriers	Drug	Key findings	Indication	Reference
	Daunorubicin + cytarabine	Increased efficiency and target damaged cells, improved liposome pharmacokinetics,reduced toxicity and enhancing treatment efficacy	Acute myeloid leukemia	(97)
	Irinotecan + fluorouracil + foli nic acid	Increased the bioavailability. Maximum plasma concentration decreased, and half-life increased.	Pancreatic adenocarcinoma	(98)
	Ascorbic acid (Vitamin C)	**Liposomal encapsulation technology**. Therefore,it delivers maximized absorption via "Smart" nano spheres. Higher bioavailability and ability to reach cells	Ischemia	(99)
Transfersom es	Insulin	Increased in vitro skin permeation	Hypoglycemia	(100)
	Ketoconazole	Antibacterial action as well as a high potential for drug delivery	Antimicrobial	(101)
	Raloxifene hydrochloride	Great potential for transdermal delivery	Osteoporosis	(102, 103)
	Sildenafil citrate	Reduced dosage administration frequency improves transdermal permeability and bioavailability.	Sexual function	(104)
	Ovalbumin and saponin	Increased peptide permeation into the skin	Anti-OVA (Ovalbumin) antibody titer in serum	(105)

Carriers	Drug	Key findings	Indication	Reference
	Diclofenac sodium	Improvement of both the efficacy and the safety of localized therapy combining the performance of painless liquid injection devices	NSAID (Non-steroidal anti-inflammatory drugs)	(106)
	Meloxicam	Resulted in a high entrapment efficiency. Transfersomes provide greater MX skin permeation	relieve pain, tenderness, swelling, and stiffness caused by osteoarthritis	(107, 108)
	Curcuma longa extract	Better for improving skin properties. incorporated in the creams could be highly beneficial as enhanced skin hydration and sebum level	Photoprotective	(109)
	Itraconazole	Optimized nanotransfersomes with lecithin: Span®60, showed the best aerosolization efficiency	Anti-fungal	(110)
	Piroxicam	Improved stability and highest elasticity in its gel formulation	NSAID (Non-steroidal anti-inflammatory drugs)	(107)
Ethosomes	5-aminolevulivic acid (ALA)	Delivery of ALA in the inflammatory skin.	Anti- psoriasis	(68, 111)
	Erythromycin	Highly efficient in eradicating S. aureus-induced intradermal infections	Antibacterial	(37)
	Minoxidil	Enhance the penetration and accumulation of minoxidil in the skin	Hair growth promoter	(112)

Carriers	Drug	Key findings	Indication	Reference
Niosomes	Aceclofenac	Improves the penetration and therapeutic efficacy of the drug, acts as a reservoir for a prolonged period and serve as a penetration enhancer.	Pain management	(113)
	Ketoprofen	Prolonged drug release, encapsulated in niosomes containing Span 60 for topical application, and was released in a slow and sustained manner.	Anti-inflammatory	(114)
	Simvastatin	Improved not only the bioavailability of the drug but also its hypocholesterolemic effect	Hypercholesterolemia	(115)
	Flurbiprofen	Afforded high drug loading and skin permeation	Ulcer treatment	(116)
	Capsaicin	Better percutaneous permeation. Diffused faster from the niosomalmatrix than from the microemulsions	Pain relief	(117)
	Salidroside	Enhanced permeation and skin deposition. Good biocompatibility with skin tissue	Neuroprotective activities	(118)
	Baclofen	Improves the low skin penetration and poor bioavailability of conventional topical formulations	Muscle relaxant	(119)

Carriers	Drug	Key findings	Indication	Reference
Ufasomes	Clotrimazole	The drug's sustained release led to the conclusion that it could be useful in the treatment of skin infections such as candidiasis.	Anti-fungal	(120)
	Minoxidil	The concentration of minoxidil gel was ten times higher than the control, indicating that it is effective in delivering drugs to the skin and follicles.	Vasodilator	(121)
	Glucose amine sulphate	Can be used as an alternative to topical anti-osteoarthritis medication	Antiosteoarthritic	(122)
	Cinnarizine	Penetrates deep nasal mucosa layer and cinnarizine loaded ufasome vesicle is possible for intranasal delivery.	Nasal infection	(75)
	Fluconazole	Penetrate stratum corneum. Potential carrier topical targeted delivery.	Anti-fungal	(123)
Sphingosomes	Beclomethasone	Enhanced penetration of drug	Skin / dermal therapy	(124)
	Sphingosomes™ Moist	Improve the low skin penetration and poor bioavailability of formulations	Skin cleansing & make-up removal efficiency	(32, 125)
	Sphingosine and sphinganine, free sphingolipids of the stratum corneum	Releases the drug at local sites of infection, reduction of drug dosage and skin irritation	Anti-fungal	(126)
	Idoxuridine	Drug entrapped inside possess optimum corneal and increase contact time	Herpatiticskeatitis	(127)

Carriers	Drug	Key findings	Indication	Reference
	Topotecan	Increases efficiency and target damaged cells, improves pharmacokinetics,reduces toxicity and enhances treatment efficacy	Treatment of lung cancer	(128)
Cubosomes	Capsaicin	Capsaicin was released continuously, skin retention was prolonged with no irritation, and capsaicin was stable under intense light and high temperatures.	Psoriasis	(129)
	Silver sulfadiazine	When compared to commercially available products, this method produces great healing results with fewer adverse effects.	Treatment of infected burns.	(130)
	Erythromycin	Effective at delivering erythromycin to the skin in a non-invasive and long-lasting method	Treatment and prevention of several types of acne	(131)
	Cyclosporine A	Cubosomes showed low ocular irritation, improved ocular bioavailability and increased precorneal retention time of cyclosporine A.	Immunosuppressive agent	(132)
	Dapsone	Cubosomes enhance permeation of dapsone across the epidermal layers at the local site, reducing systemic side effects with higher transdermal flux value compared to marketed formulation.	Antiinflammatory agent	(133)

Carriers	Drug	Key findings	Indication	Reference
	Indomethacin	Prolong the anti-inflammatory activity of the loaded, depot effect on the epidermis	Anti-inflammatory	(86)
	Flurbiprofen	Showed low ocular irritation and improved transcorneal permeation of FB.	NSAID for occular irritation	(134)
	Metformin	The cubosomes formulation significantly lowered the concentration at which viable cells were destroyed compared to metformin alone.	Anticancer	(135, 136)
	Thymoquinone	A dose and time-dependent increase in apoptotic cells was observed when treated with thymoquinone-cubosome formulation against thymoquinone alone.	Anticancer	(137)
	Losartan-Amlodipine	Preparation, Characterization and Transdermal Permeation of Losartan-Amlodipine Molecular Sal	Hypertension	(138)
	Zinc Oxide	Development and Characterization of Anticancer Model Drug Conjugated to Biosynthesized Zinc Oxide Nanoparticles Loaded into Different Topical Skin Formulations	Anticancer	(139)

III. Conclusion

In recent years, vesicular carriers have shown promise as transdermal drug delivery platforms. Compared to conventional transdermal drug delivery methods, these carriers offer a number of advantages, including improved skin permeability, enhanced drug stability, and targeted drug administration. We have examined various vesicular carriers designed for transdermal drug delivery, such as liposomes, niosomes, ethosomes, and transfersomes. Each type of carrier possesses unique advantages and qualities. For instance, niosomes are less prone to degradation by skin enzymes than liposomes, which are renowned for their ability to encapsulate and deliver both hydrophilic and hydrophobic drugs. Ethosomes and transfersomes both incorporate permeation enhancers into their carrier structure to increase skin penetration. Researchers continue to innovate in the area of vesicular carriers for transdermal drug delivery. One notable recent advancement is the development of nanostructured lipid carriers, which can transport larger drug molecules than conventional vesicular carriers. NLCs are more optimal for long-term storage due to their higher stability compared to conventional vesicular carriers. While vesicular carriers for transdermal drug delivery require further research to perfect their design and formulation, they have the potential to revolutionize the way medications are administered through the skin. Areas for potential new studies to improve the efficacy and safety of vesicular carriers for transdermal drug delivery include: the creation of novel vesicular carriers with better targeting capabilities, higher drug loading capacities, and improved stability; enhancing vesicular carrier and formulation compatibility with skin and reducing skin irritation; investigating the use of vesicular carriers for the delivery of difficult-to-dissolve medications and those susceptible to skin degradation; and conducting clinical trials to evaluate the safety and efficacy of vesicular carriers for drug delivery to treat a variety of diseases.

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REFERENCES

1. https://www.alliedmarketresearch.com/transdermal-drug-delivery-systems-market.
2. Elzainy AAW, Gu X, Simons FER, Simons KJ. Cetirizine from topical phosphatidylcholine liposomes: Evaluation of peripheral antihistaminic activity and systemic absorption in a rabbit model. *Biopharmaceutics & Drug Disposition*. 2004; 25(8): 359-66.
3. Sintov AC, Botner S. Transdermal drug delivery using microemulsion and aqueous systems: influence of skin storage conditions on the in vitro permeability of diclofenac from aqueous vehicle systems. *Int J Pharm*. 2006; 311(1-2): 55-62.
4. Pierre MB, Tedesco AC, Marchetti JM, Bentley MV. Stratum corneum lipids liposomes for the topical delivery of 5-aminolevulinic acid in photodynamic therapy of skin cancer: preparation and in vitro permeation study. *BMC Dermatol*. 2001; 1: 5.
5. Barry BW. Lipid-Protein-Partitioning theory of skin penetration enhancement. *Journal of Controlled Release*. 1991; 15(3): 237-48.
6. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. *Eur J Pharm Sci*. 2001; 14(2): 101-14.

7. Sala M, Diab R, Elaissari A, Fessi H. Lipid nanocarriers as skin drug delivery systems: Properties, mechanisms of skin interactions and medical applications. *Int J Pharm*. 2018; 535(1-2): 1-17.
8. De Jager M, Groenink W, van der Spek J, Janmaat C, Gooris G, Ponec M, et al. Preparation and characterization of a stratum corneum substitute for in vitro percutaneous penetration studies. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2006; 1758(5): 636-44.
9. De Jager MW, Gooris GS, Dolbnya IP, Ponec M, Bouwstra JA. Modelling the stratum corneum lipid organisation with synthetic lipid mixtures: the importance of synthetic ceramide composition. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2004; 1664(2): 132-40.
10. Chien YW. Novel drug delivery systems. New York: M. Dekker; 1992; 139-96.
11. Fang JY, Leu YL, Chang CC, Lin CH, Tsai YH. Lipid nano/submicron emulsions as vehicles for topical flurbiprofen delivery. *Drug Deliv*. 2004; 11(2): 97-105.
12. Mezei M, Gulasekharam V. Liposomes--a selective drug delivery system for the topical route of administration. Lotion dosage form. *Life Sci*. 1980; 26(18): 1473-7.
13. Goñi FM, Alonso A. Biophysics of sphingolipids I. Membrane properties of sphingosine, ceramides and other simple sphingolipids. *Biochim Biophys Acta*. 2006; 1758(12): 1902-21.
14. Hashizume M, Inoue H, Katagiri K, Ikeda A, Kikuchi J-I. Cerasome as an Organic-Inorganic Vesicular Nanohybrid: Characterization of Cerasome-Forming Lipids having a Single or a Dual Trialkoxysilyl Head. *Journal of Sol-Gel Science and Technology*. 2004; 31(1): 99-102.
15. Hashizume M, Kawanami S-i, Iwamoto S, Isomoto T, Kikuchi J-i. Stable vesicular nanoparticle 'Cerasome' as an organic–inorganic hybrid formed with organoalkoxysilane lipids having a hydrogen-bonding unit. *Thin Solid Films*. 2003; 438-439: 20-6.
16. Kweon JH, Chi SC, Park ES. Transdermal delivery of diclofenac using microemulsions. *Arch Pharm Res*. 2004; 27(3): 351-6.
17. Singh D, Pradhan M, Nag M, Singh MR. Vesicular system: Versatile carrier for transdermal delivery of bioactives. *Artificial Cells, Nanomedicine, and Biotechnology*. 2015; 43(4): 282-90.
18. Kumar R, Katare OP. Lecithin organogels as a potential phospholipid-structured system for topical drug delivery: a review. *AAPS PharmSciTech*. 2005; 6(2): E298-E310.
19. Park KM, Lee MK, Hwang KJ, Kim CK. Phospholipid-based microemulsions of flurbiprofen by the spontaneous emulsification process. *Int J Pharm*. 1999; 183(2): 145-54.
20. Honeywell-Nguyen PL, Bouwstra JA. Vesicles as a tool for transdermal and dermal delivery. *Drug Discov Today Technol*. 2005; 2(1): 67-74.
21. Yoshida PA, Yokota D, Foglio MA, Rodrigues RA, Pinho SC. Liposomes incorporating essential oil of Brazilian cherry (Eugenia uniflora L.): characterization of aqueous dispersions and lyophilized formulations. *J Microencapsul*. 2010; 27(5): 416-25.
22. Kulkarni SB, Betageri GV, Singh M. Factors affecting microencapsulation of drugs in liposomes. *J Microencapsul*. 1995; 12(3): 229-46.
23. Bennett WFD, MacCallum JL, Tieleman DP. Thermodynamic Analysis of the Effect of Cholesterol on Dipalmitoylphosphatidylcholine Lipid Membranes. *Journal of the American Chemical Society*. 2009; 131(5): 1972-8.
24. Jain S, Patel N, Shah MK, Khatri P, Vora N. Recent Advances in Lipid-Based Vesicles and Particulate Carriers for Topical and Transdermal Application. *J Pharm Sci*. 2017; 106(2): 423-45.

25. Gaur PK, Mishra S, Gupta VB, Rathod MS, Purohit S, Savla BA. Targeted drug delivery of Rifampicin to the lungs: formulation, characterization, and stability studies of preformed aerosolized liposome and in situ formed aerosolized liposome. *Drug Development and Industrial Pharmacy*. 2010; 36(6): 638-46.
26. New R. Liposomes : a practical approach1990. 33-104 p.
27. Modi C, Bharadia P. Transfersomes: New Dominants for Transdermal Drug Delivery. *Am J Pharm Tech Res*. 2012; 2.
28. Jain SK, Umamaheshwari RB, Bhadra D, Jain N. Ethosomes : A Novel Vesicular Carrier For Enhanced Transdermal Delivery Of An AntiHIV Agent. *Indian Journal of Pharmaceutical Sciences*. 2004; 66: 72-81.
29. Kazi KM, Mandal AS, Biswas N, Guha A, Chatterjee S, Behera M, et al. Niosome: A future of targeted drug delivery systems. *J Adv Pharm Technol Res*. 2010; 1(4): 374-80.
30. Naik A, Pechtold LARM, Potts RO, Guy RH. Mechanism of oleic acid-induced skin penetration enhancement in vivo in humans. *Journal of Controlled Release*. 1995; 37(3): 299-306.
31. Saraf PS, Gupta D, Kaur DCD, Saraf S. Sphingosomes a novel approach to vesicular drug delivery. *Int J Cur Sci Res*. 2011; 1: 63-8.
32. Spicer PT, Hayden K, Lynch ML, Ofori-Boateng, Akua, Burns JL. Novel Process for Producing Cubic Liquid Crystalline Nanoparticles (Cubosomes). *Langmuir*. 2001; 17: 5748-56.
33. Walve JR, Bakliwal SR, Rane B, Pawar SP. Transfersomes: A surrogated carrier for transdermal drug delivery system. *Int J Appl Biol Pharm Technol*. 2011; 2: 204-13.
34. Reddy D, Sravani A. Transferosomes A Novel Vesicular Carrier for Transdermal Drug Delivery System. 2015: 193-208.
35. Maestrelli F, Capasso G, González-Rodríguez ML, Rabasco AM, Ghelardini C, Mura P. Effect of preparation technique on the properties and in vivo efficacy of benzocaine-loaded ethosomes. *J Liposome Res*. 2009; 19(4): 253-60.
36. Zahid S, Upmanyu N, Dangi S, Ray S, Jain P, Parkhe G. Ethosome: a novel vesicular carrier for transdermal drug delivery. *Journal of Drug Delivery and Therapeutics*. 2018; 8: 318-26.
37. David SR, Hui MS, Pin CF, Ci FY, Rajabalaya R. Formulation and in vitro evaluation of ethosomes as vesicular carrier for enhanced topical delivery of isotretinoin. *International Journal of Drug Delivery*. 2013; 5: 28-34.
38. Song CK, Balakrishnan P, Shim CK, Chung SJ, Chong S, Kim DD. A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: characterization and in vitro/in vivo evaluation. *Colloids Surf B Biointerfaces*. 2012; 92: 299-304.
39. Zhang JP, Wei YH, Zhou Y, Li YQ, Wu XA. Ethosomes, binary ethosomes and transfersomes of terbinafine hydrochloride: a comparative study. *Arch Pharm Res*. 2012; 35(1): 109-17.
40. Ainbinder D, Godin B, Touitou E. Ethosomes: Enhanced Delivery of Drugs to and Across the Skin. In: Dragicevic N, Maibach HI, editors. Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Nanocarriers. Berlin, Heidelberg: Springer Berlin Heidelberg; 2016; 61-75.
41. Md U, Ghuge P, Jain B. Niosomes: A Novel Trend of Drug Delivery. *European Journal of Biomedical and Pharmaceutical sciences (EJBPS).* 2017.
42. Hu C, Rhodes DG. Proniosomes: a novel drug carrier preparation. *Int J Pharm*. 1999; 185(1): 23-35.
43. Auda SH, Fathalla D, Fetih G, El-Badry M, Shakeel F. Niosomes as transdermal drug delivery system for celecoxib: in vitro and in vivo studies. *Polymer Bulletin*. 2016; 73(5): 1229-45.

44. Muzzalupo R, Tavano L, Cassano R, Trombino S, Ferrarelli T, Picci N. A new approach for the evaluation of niosomes as effective transdermal drug delivery systems. *Eur J Pharm Biopharm*. 2011; 79(1): 28-35.
45. Mittal R, Sharma A, Arora S. Ufasomes Mediated Cutaneous Delivery of Dexamethasone: Formulation and Evaluation of Anti-Inflammatory Activity by Carrageenin-Induced Rat Paw Edema Model. *J Pharm (Cairo)*. 2013; 2013: 680580.
46. Patel DM, Patel CN, Jani R. Ufasomes: A vesicular drug delivery. *Systematic Reviews in Pharmacy*. 2011; 2.
47. Nair AJ, Aswathi K, George A, Athira PP, Nair SC. UFASOME: A POTENTIAL PHOSPHOLIPID CARRIER AS A NOVEL PHARMACEUTICAL FORMULATION. *International research journal of pharmacy*. 2014; 5: 250-3.
48. Desu P. SPHINGOSOMES: A NOVEL VESICULAR DRUG DELIVERY SYSTEM. 2013; 305-12.
49. Witika BA, Mweetwa LL, Tshiamo KO, Edler K, Matafwali SK, Ntemi PV, et al. Vesicular drug delivery for the treatment of topical disorders: current and future perspectives. *Journal of Pharmacy and Pharmacology*. 2021; 73(11): 1427-41.
50. Bei D, Meng J, Youan BB. Engineering nanomedicines for improved melanoma therapy: progress and promises. Nanomedicine (Lond). 2010; 5(9): 1385-99.
51. Salah S, Mahmoud AA, Kamel AO. Etodolac transdermal cubosomes for the treatment of rheumatoid arthritis: ex vivo permeation and in vivo pharmacokinetic studies. *Drug Delivery*. 2017; 24(1): 846-56.
52. Nasr M, Younes H, Abdel-Rashid RS. Formulation and evaluation of cubosomes containing colchicine for transdermal delivery. *Drug Delivery and Translational Research*. 2020; 10(5): 1302-13.
53. Anwekar H, Patel S, Singhai A. Liposome-as Drug Carriers. *International Journal of Pharmacy and Life Sciences*. 2011; 2: 945-51.
54. Moghimipour E, Handali S. Liposomes as Drug Delivery Systems: Properties and Applications. *Research Journal of Pharmaceutical, Biological and Chemical*. 2013; 4(1): 169-85.
55. Characterization of the final nanoformulation product. (New R.C.C. Preparation of liposomes. In: New R.C.C., editor. Liposomes: A Practical Approach. Oxford University Press; New York, NY, USA: 1990; 33–104.
56. Wu J, Liu Q, Lee RJ. A folate receptor-targeted liposomal formulation for paclitaxel. *Int J Pharm*. 2006; 316(1-2): 148-53.
57. Perez AP, Altube MJ, Schilrreff P, Apezteguia G, Celes FS, Zacchino S, et al. Topical amphotericin B in ultradeformable liposomes: Formulation, skin penetration study, antifungal and antileishmanial activity in vitro. *Colloids Surf B Biointerfaces*. 2016; 139: 190-8.
58. Cevc G, Blume G. New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers, Transfersomes. *Biochim Biophys Acta*. 2001; 1514(2): 191-205.
59. Sharma N, Aggarwal G, Rana AC, Bhat Z, Kumar D. A Review: Transdermal Drug Delivery System: A Tool For Novel Drug Delivery System. *International Journal of Drug Development and Research*. 2011; 3: 70-84.
60. Patel R, Baria AH. Formulation and evaluation considerations of transdermal drug delivery system. *International Journal of Pharmaceutical Research*. 2011; 3: 1-9.
61. Monika B, Roy A, Bahadur S, Alisha B, Mihir P, Dhanushram T. Transdermal drug delivery system with formulation and evaluation aspects: Overview. *Research Journal of Pharmacy and Technology*. 2012; 5: 1168-76.
62. Paudel KS, Milewski M, Swadley CL, Brogden NK, Ghosh P, Stinchcomb AL. Challenges and opportunities in dermal/transdermal delivery. *Ther Deliv*. 2010; 1(1): 109-31.

63. Opatha SAT, Titapiwatanakun V, Chutoprapat R. Transfersomes: A Promising Nanoencapsulation Technique for Transdermal Drug Delivery. Pharmaceutics. 2020; 12(9): 855. doi: 10.3390/pharmaceutics12090855
64. Touitou E, Godin B, Weiss C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. *Drug Development Research*. 2000; 50(3-4): 406-15.
65. López-Pinto JM, González-Rodríguez ML, Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. *Int J Pharm*. 2005; 298(1): 1-12.
66. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes — novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *Journal of Controlled Release*. 2000; 65(3): 403-18.
67. Touitou E, Godin B. Ethosomes for skin delivery. *Journal of Drug Delivery Science and Technology*. 2007; 17(5): 303-8.
68. Abdelkader H, Alani AW, Alany RG. Recent advances in non-ionic surfactant vesicles (niosomes): self-assembly, fabrication, characterization, drug delivery applications and limitations. *Drug Deliv*. 2014; 21(2): 87-100.
69. Yadav, Kumar S, Mishra M, Garima V, Nayak K, Tiwari A, et al. NIOSOMES: AN APPROACH TOWARDS TARGETED DRUG DELIVERY SYSTEM. *International Journal of Institutional Pharmacy and Life Sciences*. 2016; 6: 39-52.
70. G DB, P VL. Recent advances of non-ionic surfactant-based nano-vesicles (niosomes and proniosomes): a brief review of these in enhancing transdermal delivery of drug. *Future Journal of Pharmaceutical Sciences*. 2020; 6(1): 100.
71. Zaid Alkilani A, Hamed R, Abdo H, Swellmeen L, Basheer HA, Wahdan W, Abu Kwiak AD. Formulation and Evaluation of Azithromycin-Loaded Niosomal Gel: Optimization, In Vitro Studies, Rheological Characterization, and Cytotoxicity Study. *ACS Omega*. 2022; 7 (44): 39782-39793. doi: 10.1021/acsomega.2c03762. PMID: 36385887; PMCID: PMC9648136.
72. Schreier H, Bouwstra J. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. *Journal of Controlled Release*. 1994; 30(1): 1-15.
73. Yu Y-Q, Yang X, Wu X-F, Fan Y-B. Enhancing Permeation of Drug Molecules Across the Skin via Delivery in Nanocarriers: Novel Strategies for Effective Transdermal Applications. *Frontiers in Bioengineering and Biotechnology*. 2021; 9.
74. Salama AH, Aburahma MH. Ufasomes nano-vesicles-based lyophilized platforms for intranasal delivery of cinnarizine: preparation, optimization, ex-vivo histopathological safety assessment and mucosal confocal imaging. *Pharm Dev Technol*. 2016; 21(6): 706-15.
75. Kanikkannan N, Kandimalla K, Lamba SS, Singh M. Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. *Curr Med Chem*. 2000; 7(6): 593-608.
76. Singh S, Malik BK, Sharma DK. Molecular drug targets and structure based drug design: A holistic approach. *Bioinformation*. 2006; 1(8): 314-20.
77. Gaur PK, Mishra S, Verma A, Verma N. Ceramide–palmitic acid complex based Curcumin solid lipid nanoparticles for transdermal delivery: pharmacokinetic and pharmacodynamic study. *Journal of Experimental Nanoscience*. 2016; 11(1): 38-53.

78. Gaur PK, Purohit S, Kumar Y, Mishra S, Bhandari A. Ceramide-2 nanovesicles for effective transdermal delivery: development, characterization and pharmacokinetic evaluation. *Drug Development and Industrial Pharmacy*. 2014; 40(4): 568-76.
79. Jain S, Jain V, Mahajan SC. Lipid Based Vesicular Drug Delivery Systems. *Advances in Pharmaceutics*. 2014; 2014: 574673.
80. Stefanov SR, Andonova VY. Lipid Nanoparticulate Drug Delivery Systems: Recent Advances in the Treatment of Skin Disorders. *Pharmaceuticals (Basel).* 2021; 14(11).
81. S. T. Prajapati, C. G. Patel, and C. N. Patel. Transfersomes: a vesicular carrier system for transdermal drug delivery. *Asian Journal of Biochemical and Pharmaceutical Research*. 2021; 2(1): 507–524.
82. Anbarasan, Grace F, editors. AN OVERVIEW OF CUBOSOMES-SMART DRUG DELIVERY SYSTEM. 2016.
83. Spicer PT, editor Bicontinuous Cubic Liquid Crystalline Phase and Cubosome + Personal Care Delivery Systems. 2003.
84. Tilekar K, Khade PK, Kakade S, Kotwal S, editors. CUBOSOMES-A DRUG DELIVERY SYSTEM.2014.
85. Esposito E, Cortesi R, Drechsler M, Paccamiccio L, Mariani P, Contado C, et al. Cubosome dispersions as delivery systems for percutaneous administration of indomethacin. *Pharm Res*. 2005; 22(12): 2163-73.
86. Nasr M, Ghorab MK, Abdelazem A. In vitro and in vivo evaluation of cubosomes containing 5-fluorouracil for liver targeting. *Acta Pharmaceutica Sinica B*. 2015; 5(1): 79-88.
87. Flak DK, Adamski V, Nowaczyk G, Szutkowski K, Synowitz M, Jurga S, et al. AT101-Loaded Cubosomes as an Alternative for Improved Glioblastoma Therapy. *Int J Nanomedicine*. 2020; 15: 7415-31.
88. Nanjwade B, Hundekar Y, Kamble M, Srichana T. Development of Cuboidal Nanomedicine by Nanotechnology. *Development of Cuboidal Nanomedicine by Nanotechnology*. 2014; 2: 1-8.
89. Naeff R. Feasibility of topical liposome drugs produced on an industrial scale. *Advanced Drug Delivery Reviews*. 1996; 18(3): 343-7.
90. Sand M, Bechara FG, Sand D, Altmeyer P, Hoffmann K. A randomized, controlled, double-blind study evaluating melanin-encapsulated liposomes as a chromophore for laser hair removal of blond, white, and gray hair. *Ann Plast Surg*. 2007; 58(5): 551-4.
91. Foldvari M. In vitro cutaneous and percutaneous delivery and in vivo efficacy of tetracaine from liposomal and conventional vehicles. *Pharm Res*. 1994; 11(11): 1593-8.
92. Hofland HE, van der Geest R, Bodde HE, Junginger HE, Bouwstra JA. Estradiol permeation from nonionic surfactant vesicles through human stratum corneum in vitro. *Pharm Res*. 1994; 11(5): 659-64.
93. Cevc G, Vierl U, Mazgareanu S. Functional characterisation of novel analgesic product based on self-regulating drug carriers. *Int J Pharm*. 2008; 360(1-2): 18-28.
94. Senapati S, Mahanta AK, Kumar S, Maiti P. Controlled drug delivery vehicles for cancer treatment and their performance. *Signal Transduction and Targeted Therapy*. 2018; 3(1): 7.
95. Deitcher OR, Glaspy J, Gonzalez R, Sato T, Bedikian AY, Segarini K, et al. High-dose Vincristine Sulfate Liposome Injection (Marqibo) Is Not Associated With Clinically Meaningful Hematologic Toxicity. *Clinical Lymphoma Myeloma and Leukemia*. 2014; 14(3): 197-202.
96. Din FU, Aman W, Ullah I, Qureshi OS, Mustapha O, Shafique S, et al. Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *Int J Nanomedicine*. 2017; 12: 7291-309.

97. Pelzer U, Blanc JF, Melisi D, Cubillo A, Von Hoff DD, Wang-Gillam A, et al. Quality-adjusted survival with combination nal-IRI+5-FU/LV vs 5-FU/LV alone in metastatic pancreatic cancer patients previously treated with gemcitabine-based therapy: a Q-TWiST analysis. *Br J Cancer*. 2017; 116(10): 1247-53.
98. Davis JL, Paris HL, Beals JW, Binns SE, Giordano GR, Scalzo RL, et al. Liposomal-encapsulated Ascorbic Acid: Influence on Vitamin C Bioavailability and Capacity to Protect Against Ischemia-Reperfusion Injury. *Nutr Metab Insights*. 2016; 9: 25-30.
99. Cevc G. Transdermal drug delivery of insulin with ultradeformable carriers. *Clin Pharmacokinet*. 2003; 42(5): 461-74.
100. Cevc G, Gebauer D, Stieber J, Schätzlein A, Blume G. Ultraflexible vesicles, Transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. *Biochim Biophys Acta*. 1998; 1368(2): 201-15.
101. Opatha SAT, Titapiwatanakun V, Chutoprapat R. Transfersomes: A Promising Nanoencapsulation Technique for Transdermal Drug Delivery. *Pharmaceutics*. 2020; 12(9): 855.
102. Singh S, Verma D, Mirza MA, Das AK, dudeja M, Anwer MK, et al. Development and optimization of ketoconazole loaded nano-transfersomal gel for vaginal delivery using Box-Behnken design: In vitro, ex vivo characterization and antimicrobial evaluation. *Journal of Drug Delivery Science and Technology*. 2017; 39: 95-103.
103. Mahmood S, Chatterjee B, Mandal UK, editors. Nano transfersomes vesicles of raloxifene HCl with sorbitan 80: formulation and characterization. 2018.
104. Zhang Y, Ng W, Feng X, Cao F, Xu H. Lipid vesicular nanocarrier: Quick encapsulation efficiency determination and transcutaneous application. *International Journal of Pharmaceutics*. 2017; 516(1-2): 225-30.
105. Schlich M, Lai F, Murgia S, Valenti D, Fadda AM, Sinico C. Needle-free jet injection of intact phospholipid vesicles across the skin: a feasibility study. *Biomed Microdevices*. 2016; 18(4): 67.
106. Garg V, Singh H, Bhatia A, Raza K, Singh SK, Singh B, et al. Systematic Development of Transethosomal Gel System of Piroxicam: Formulation Optimization, In *Vitro Evaluation, and Ex Vivo Assessment. AAPS PharmSciTech*. 2017; 18(1): 58-71.
107. Duangjit S, Opanasopit P, Rojanarata T, Ngawhirunpat T. Evaluation of meloxicam-loaded cationic transfersomes as transdermal drug delivery carriers. *AAPS PharmSciTech*. 2013; 14(1): 133-40.
108. Kaur CD, Saraf S. Topical vesicular formulations of Curcuma longa extract on recuperating the ultraviolet radiation-damaged skin. *J Cosmet Dermatol*. 2011; 10(4): 260-5.
109. Hassanpour Aghdam M, Ghanbarzadeh S, Javadzadeh Y, Hamishehkar H. Aggregated Nanotransfersomal Dry Powder Inhalation of Itraconazole for Pulmonary Drug Delivery. *Adv Pharm Bull*. 2016; 6(1): 57-64.
110. Fang Y-P, Huang Y-B, Wu P-C, Tsai Y-H. Topical delivery of 5-aminolevulinic acid-encapsulated ethosomes in a hyperproliferative skin animal model using the CLSM technique to evaluate the penetration behavior. *European Journal of Pharmaceutics and Biopharmaceutics*. 2009; 73(3): 391-8.
111. Sudhakar K, Nitish U SJR, Narayana C. Ethosomes as Non-invasive Loom for Transdermal Drug Delivery System. 2012; 1-22.
112. Solanki AB, Parikh JR, Parikh RH, Patel MR, editors. Evaluation of different compositions of niosomes to optimize aceclofenac transdermal delivery. 2010.
113. Arora R, Sharma A. Release Studies of Ketoprofen Niosome Formulation. *J Chem Pharm Res*. 2010; 2.

114. Shaker D, Nasr M, Mostafa M. Bioavailability and hypocholesterolemic effect of proniosomal simvastatin for transdermal delivery. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2013; 5: 344-51.
115. Ibrahim MMA, Sammour OA, Hammad MA, Megrab NA. In vitro evaluation of proniosomes as a drug carrier for flurbiprofen. *AAPS PharmSciTech*. 2008; 9(3): 782-90.
116. Tavano L, Alfano P, Muzzalupo R, de Cindio B. Niosomes vs microemulsions: new carriers for topical delivery of Capsaicin. Colloids Surf B Biointerfaces. 2011; 87(2): 333-9.
117. Zhang Y, Zhang K, Wu Z, Guo T, Ye B, Lu M, et al. Evaluation of transdermal salidroside delivery using niosomes via in vitro cellular uptake. *Int J Pharm*. 2015; 478(1): 138-46.
118. Keservani R, Sharma AK, Ramteke S. Novel Vesicular Approach For Topical Delivery of Baclofen Via Niosomes. *Latin American Journal of Pharmacy*. 2010; 29: 1364-70.
119. Bolla PK, Meraz CA, Rodriguez VA, Deaguero I, Singh M, Yellepeddi VK, et al. Clotrimazole Loaded Ufosomes for Topical Delivery: Formulation Development and In-Vitro Studies. *Molecules*. 2019; 24(17).
120. Kumar P, Singh SK, Handa V, Kathuria H. Oleic Acid Nanovesicles of Minoxidil for Enhanced Follicular Delivery. *Medicines (Basel).* 2018; 5(3): 103.
121. Arundhasree, R R, R A, Kumar A, Kumar S, Nair S. UFASOMES: UNSATURATED FATTY ACID BASED VESICULAR DRUG DELIVERY SYSTEM. *International Journal of Applied Pharmaceutics*. 2021; 76-83.
122. Zakir F, Vaidya B, Goyal AK, Malik B, Vyas SP. Development and characterization of oleic acid vesicles for the topical delivery of fluconazole. *Drug Delivery*. 2010; 17(4): 238-48.
123. Sreekanth DN. SPHINGOSOMES: A NOVEL VESICULAR DRUG DELIVERY SYSTEM. *International Journal of Pharmaceutical Research and Bio Science*. 2013; 2: 305-12.
124. Mathew B, Jacobson JR, Berdyshev E, Huang Y, Sun X, Zhao Y, et al. Role of sphingolipids in murine radiation-induced lung injury: protection by sphingosine 1-phosphate analogs. *Faseb j*. 2011; 25(10): 3388-400.
125. Baumruker T, Prieschl EE. Sphingolipids and the regulation of the immune response. *Semin Immunol*. 2002; 14(1): 57-63.
126. Kunwarpuriya AS, Doke V, Changedia S, Khutle NM, editors. SPHINGOSOME: A NOVEL VESICULAR DRUG DELIVERY SYSYTEM. 2015.
127. Mui BL-S, Ahkong Q-F, Leone R, Wong K, Lam K, Chow L, et al. Encapsulation of crystalline topotecan in sphingosomes: a formulation suitable for clinical development. *Cancer Research*. 2005; 65 (9_Supplement): 326-7.
128. Abourehab MAS, Ansari MJ, Singh A, Hassan A, Abdelgawad MA, Shrivastav P, et al. Cubosomes as an emerging platform for drug delivery: a review of the state of the art. *Journal of Materials Chemistry B*. 2022; 10(15): 2781-819.
129. Morsi NM, Abdelbary GA, Ahmed MA. Silver sulfadiazine based cubosome hydrogels for topical treatment of burns: development and in vitro/in vivo characterization. *Eur J Pharm Biopharm*. 2014; 86(2): 178-89.
130. Khan S, Jain P, Jain S, Jain R, Bhargava S, Jain A. Topical Delivery of Erythromycin Through Cubosomes for Acne. *Pharm Nanotechnol*. 2018; 6(1): 38-47.
131. Lai J, Lu Y, Yin Z, Hu F, Wu W. Pharmacokinetics and enhanced oral bioavailability in beagle dogs of cyclosporine A encapsulated in glyceryl monooleate/poloxamer 407 cubic nanoparticles. *Int J Nanomedicine*. 2010; 5: 13-23.

132. Nithya R, Jerold P, Siram K. Cubosomes of dapsone enhanced permeation across the skin. *Journal of Drug Delivery Science and Technology*. 2018; 48: 75-81.
133. Han S, Shen JQ, Gan Y, Geng HM, Zhang XX, Zhu CL, et al. Novel vehicle based on cubosomes for ophthalmic delivery of flurbiprofen with low irritancy and high bioavailability. *Acta Pharmacol Sin*. 2010; 31(8): 990-8.
134. Saber MM, Al-Mahallawi AM, Nassar NN, Stork B, Shouman SA. Targeting colorectal cancer cell metabolism through development of cisplatin and metformin nano-cubosomes. BMC Cancer. 2018; 18(1): 822.
135. Magdy M, Almahallawi A, Nassar N, Shouman S. Pluronic Based Cubosomes Enhance Metformin Cytotoxicity In Colon Cancer Cell Lines. *Clinical Therapeutics*. 2017; 39(8, Supplement): e27.
136. Mehanna MM, Sarieddine R, Alwattar JK, Chouaib R, Gali-Muhtasib H. Anticancer Activity of Thymoquinone Cubic Phase Nanoparticles Against Human Breast Cancer: Formulation, Cytotoxicity and Subcellular Localization. *Int J Nanomedicine*. 2020; 15: 9557-70.
137. Varghese R, Salvi S, Sood P, Kulkarni B, Kumar D. Cubosomes in cancer drug delivery: A review. *Colloid and Interface Science Communications*. 2022; 46: 100561.
138. Al Khawaja AY, Khalil EA, Mansour RSH, Hamdan II. Preparation, Characterization and Transdermal Permeation of Losartan-Amlodipine Molecular Salt. *Jordan Journal of Pharmaceutical Sciences*. 2022; 15(4): 536-52.
139. Obaid RZ, Abu-Huwaij R, Hamed R. Development and Characterization of Anticancer Model Drug Conjugated to Biosynthesized Zinc Oxide Nanoparticles Loaded into Different Topical Skin Formulations. *Jordan Journal of Pharmaceutical Sciences*. 2023; 16(2): 486.

التطورات الحديثة في تطوير الناقل الحويصلي لتوصيل الأدوية عبر الجلد: مراجعة

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ملخص

بالنسبة للباحثين في مجال الأدوية، أثار أسلوب إعطاء الدواء عبر الجلد اهتمامهم. تعتبر الطبقة القرنية، وهي الطبقة الخارجية للجلد، عائقًا رئيسيًا أمام انتشار الأدوية والمواد النشطة بيولوجيًا عبر الطرق عبر الجلد. يتم الآن استخدام أساليب مختلفة، مثل الأساليب الفيزيائية، والطرق الكيميائية، وناقلات التسليم، لتحسين التوصيل عبر الجلد النشط بيولوجيًا. تقدم هذه المراجعة لمحة موجزة عن الجلد، وآليات نقل الأدوية عبر الجلد، بالإضافة إلى الأنظمة الحويصلية الدهنية المختلفة، مع التركيز على الأنظمة الحويصلية الدهنية مثل الجسيمات الناقلة، والجسيمات الشحمية، والنيوزومات، والإيثوسومات، والجسيمات اليوفا، والجسيمات السفينجوزومية، والجسيمات المكعبة، ومزاياها. في التسليم والبيانات الخاصة بالعديد من التركيبات الحويصلية للتوزيع عبر الجلد تم تلخيصها في هذه المراجعة بناءً على الأبحاث المنشورة في السنوات العشر الماضية. آفاق أحدث الأساليب القائمة على المواد الطبيعية في المستقبل.

الكلمات الدالة: الجسيمات الناقلة، الجسيمات الشحمية، النيوسومات، الإيثوسومات، الجسيمات اليوفا، الجسيمات السفينجوزومية والمكعبات، توصيل الأدوية عبر الجلد، التركيبة الحويصلية.

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Navigating Changes in Patient Drug and Non-Drug Item Demands in Community Pharmacies Amidst the COVID-19 Pandemic

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ABSTRACT

During the COVID-19 pandemic, healthcare systems worldwide faced unprecedented challenges, with pharmacists playing a crucial role on the frontlines. They encountered a surge in patient requests for drugs and non-drug items related to the treatment and prevention of the disease. This cross-sectional survey aimed to assess changes in demand for selected drugs and non-drug items from the perspective of pharmacists in Jordan in the period from March to June 2021, and to explore the factors influencing this demand. An online questionnaire targeting pharmacists working in community pharmacies was developed, validated, and disseminated using social media (e.g., WhatsApp, Facebook, and Messenger). The study collected 390 responses from pharmacists working in community pharmacies. The findings revealed significant increases in demand for prescription drugs such as antibiotics (97.4%), antithrombotics (84.1%), and antivirals (66.2%), often without prescriptions. Non-prescription items, specifically minerals and vitamins, were highly sought after (100%). Demand also rose for non-drug items such as thermometers (89.0%), oximeters (85.1%) and oxygen concentrators (68.2%). Changes in drug requirements were consistent across Jordan's districts. However, an association was observed between the increase in demand for antivirals and herbal supplements and chain pharmacies (p-value 0.037 and p-value <0.005, respectively). In conclusion, COVID-19 led to a significant upsurge in the demand for pharmaceutical products and devices, placing immense pressure on community pharmacies. The public's reaction to the pandemic, to combat and manage the disease, was consistent across Jordan, regardless of social, financial, and spatial differences among the population. The study highlights the importance of adequately preparing and educating pharmacists to provide accurate information and counseling to patients in such circumstances. Therefore, health authorities must ensure that pharmacists have access to the latest treatments and management protocols and provide clear guidance on using home treatment devices to the public.

Keywords: Jordan, COVID-19, pharmacists, community pharmacy, drug demand, self-medication.

INTRODUCTION

Globally, healthcare systems have been confronted with the unprecedented challenges of the Coronavirus Disease-19 (COVID-19) pandemic since late 2019 (1). Many severe cases of COVID-19 require hospitalization, admission to intensive care units, or may even result in death (2). The pandemic has exerted enormous pressure on healthcare systems worldwide, necessitating the recruitment of all available healthcare resources and professionals to curb the spread of the virus. In response, the World Health Organization (WHO) has issued

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guidelines for the general public on managing mild to moderate cases of COVID-19 at home (3).

Throughout the pandemic, various reports have recommended certain drugs for treating or alleviating symptoms of COVID-19. Some of these treatments were adopted by health authorities, but over time, a few drugs were proven ineffective. Among the suggested drugs were hydroxychloroquine, azithromycin, paracetamol, nonsteroidal anti-inflammatory drugs, and vitamins and minerals including vitamins C and D, and zinc (4-6). Consequently, the lack of a definitive COVID-19 treatment, coupled with high death rates and claims of identifying effective treatments and immune-boosting supplements (7), have led people to seek different preventive measures (4). Simultaneously, self-medication has conspiculously increased during the pandemic in many parts of the world, with Jordan being no exception (4-6).

Under these circumstances, pharmacists have found themselves on the frontlines, dealing with a surge in patients seeking drugs for the treatment of suspected COVID-19 symptoms or for disease prevention (8). In fact, international organizations such as the International Pharmaceutical Federation (FIP) and the American Pharmacist Association (APhA) have issued guidelines to strengthen the roles of pharmacists as frontline healthcare workers during the pandemic (9,10).

Given these circumstances, it is expected that Jordanians, like people in many other countries, will also turn to self-medication during the pandemic. In the literature, several studies have reported self-medication by Jordanians using different drug classes (11-13). However, only a few studies have addressed the impact of COVID-19 on the utilization of drugs and non-drug items among Jordanians. One study investigated the prevalence and predictors of self-medication for certain drug items, as perceived by patients (14). Another study examined the effect of COVID-19 on national antimicrobial consumption, with data on antimicrobial drugs collected from the Jordan Food and Drug Administration (15). However, it is important to note that no study has yet examined the changes in the consumption and use of drugs and non-drug items from the perspective of pharmacists.

Therefore, this study aims to evaluate the changes in demand for selected drugs and non-drug items in Jordan during the COVID-19 pandemic, based on the perspectives of pharmacists. Additionally, the study seeks to determine whether the demand for these drugs was based on medical prescriptions or self-medication. Furthermore, the study aims to investigate the factors affecting drug demand during the pandemic. This information could help decision-makers understand public behaviors during pandemics and set up necessary measures and policies for future preparedness for epidemics.

METHODS

Study design and instrument

This cross-sectional study was conducted from March to June 2021 during the COVID-19 pandemic. The study used a questionnaire, with pharmacists working in community pharmacies in Jordan as the target audience. The endpoint of this study was to assess whether there was a change (increase or decrease) in the demand for selected drugs and non-drug items during the COVID-19 pandemic in Jordan based on the pharmacists' perspectives. A structured questionnaire was developed based on an extensive review of published research in the same field (5,6,14,16,17). The first page of the questionnaire provided information about the nature and objectives of the study, as well as the inclusion criteria for participation. The inclusion criteria included: (a) working in a community pharmacy during the COVID-19 pandemic, and (b) providing consent to participate in the study. To ensure participants fulfilled the inclusion criteria, two questions were added at the beginning of the survey: 'Have you been working in a community pharmacy during the COVID-19 pandemic?' and 'Do you agree to participate in the study?'. Answering 'No' to any of these questions prevented the participant from completing the survey. The

questionnaire also included a statement assuring participants of the confidentiality of their participation. The questionnaire consisted of two main sections. The first section collected demographic and general characteristics of the pharmacists (Table 1), as well as information about the community pharmacies, such as type of pharmacy and geographical location.

The second section of the questionnaire assessed changes in the demand for different drug categories and non-drug items from the pharmacists' perspectives. The participants were asked to indicate whether they observed a considerable increase, decrease, or no change in the demand for selected drug categories, or non-drug items (e.g., supplements, minerals, and devices, among others) during the COVID-19 pandemic, from a list provided to them (Tables 2 and 3). Regarding drug categories, the participants were asked to specify which class/drug of those categories had shown an increase in demand (Table 2). The questionnaire was designed in Arabic, the native language of the country, and the medication classes were written in both Arabic and English. The questionnaire underwent a thorough review by an academician, a clinical pharmacist, and a community pharmacist. For further clarification and modification of the questionnaire, it was pilot tested with 10 community pharmacists, who were excluded from the statistical analysis.

The questionnaire link was disseminated through various social media platforms for pharmacists, including Facebook®, Facebook Messenger®, and WhatsApp®. The snowball sampling technique was used to distribute the Google form and enroll participants (18,19).

Statistical analysis

A minimum sample size of 385 pharmacists was estimated as appropriate, based on the study by Zucco et al, 2018 (20). Statistical analysis was performed using SPSS version 23 (SPSS Inc., Chicago, IL). Descriptive statistics were used to summarize the demographic characteristics of the participants.

The Chi-square test and Fisher's exact test were utilized to find associations between patterns of use for different drug categories and categorical variables. Hypothesis testing was two-sided, and a p-value of < 0.05 was considered significant.

Ethical consideration

The study protocol was approved by the Institutional Review Board of the Deanship of Academic Research at the University of Jordan (IRB No. 20/2021).

RESULTS

Demographic and General Characteristics of Pharmacists

Three hundred and ninety pharmacists completed the entire questionnaire. The majority of respondents were female pharmacists (63.8%, n=249), and approximately half of them had less than five years of experience (53.6%, n=209). The mean age of respondents was 31.3 years (SD=9.2). Three-quarters (74.4%, n=290) of the pharmacies were independent, and the majority were located in the central district of Jordan (81.8%, n=319).

The demographic characteristics of the participants and the location of the pharmacies are summarized in Table 1.

Table 1 Characteristics of participants in the study (N=390)

Variable	% (n)
Age [mean±SD]	31.3 ±9.2
Gender	
Males	36.2 (141)
Females	63.8 (249)
Pharmacy type	
Chain pharmacy	25.6 (100)
Independent pharmacy	74.4 (290)
Geographical location of community pharmacy	
Central of Jordan	81.8 (319)
Amman, the capital city of Jordan	65.9 (357)
Madaba	2.6 (10)
Zarqa	6.7 (26)
Balqa	6.7 (26)
North of Jordan	15.1 (59)
South of Jordan	3.1 (12)
Years of experience	
<1 year	13.6 (53)
1-5 years	40.0 (156)
6-10 years	21.0 (82)
11-15 years	8.5 (33)
16-20 years	8.5 (33)
21-25 years	3.1 (12)
>25 years	5.4 (21)

Patterns of drug demand

The perceived increase in the demand for different drug classes is summarized in Table 2. Among the prescription drugs, antibiotics reported the highest increase in demand (97.4%, n=380), followed by antithrombotics (84.1%, n=328).

The highest increase in demand for antibiotics (Table 2) was seen for macrolides (95.1%, n=371), followed by fluoroquinolones (37.2%, n=145). Among the antithrombotic drugs, aspirin recorded the highest increase in demand (79.7%, n=311), followed by enoxaparin (31.5%, n=123).

Favipiravir was the most demanded antiviral drug during the study period (58.7%, n=229).

Minerals and vitamins reported the highest increase in demand among non-prescription drugs (100%, n=390), followed by analgesics and antipyretics (96.9%, n=378), common cold preparations (84.9%, n=331), and herbals and supplements (80.5%, n=314) (Table 2). Both zinc and vitamin C recorded the highest increase in drug demand (98.5%, n=384 and 97.7%, n=381 respectively), followed by vitamin D (88.7%, n=346). Among analgesics and antipyretics, paracetamol (96.9%, n=374) recorded the highest increase in demand. Further details are shown in Table 2.

Table 2. The pattern of drug demand during COVID-19 in Jordan as perceived by pharmacists (N=390), focusing on individual/classes of drugs or non-drug items that showed an increase in the demand

	% (n)
Antibiotics	
The demand did not change.	2.6 (10)
The demand increased significantly:	97.4 (380)
• Macrolides such as azithromycin	95.1 (371)
• Penicillins (amoxicillin, ampicillin, oxacillin, etc)	15.4 (60)
• Oral Cephalosporins (Cephalexin, cefaclor, cefixime, cefuroxime, etc..)	7.2 (28)
• Parenteral cephalosporins, (cefotaxime, ceftriaxone, cefepime, etc)	7.4 (29)
• Fluoroquinolones (Ciprofloxacin, levofloxacin, moxifloxacin, etc)	37.2 (145)
• Doxycycline	3.1 (12)
Antivirals	
The demand did not change.	33.8 (132)
The demand increased significantly:	66.2 (258)
• Oseltamivir (trade name: Tamiflu)	6.7 (26)
• Favipiravir (Sancovir)	58.7 (229)
• Remdesivir (Veklury	9.2 (36)
Analgesics and antipyretics	
The demand did not change.	3.1 (12)
The demand increased significantly:	96.9 (378)
• Oral paracetamol	95.9 (374)
• Oral ibuprofen	18.5 (72)
• Oral diclofenac (tablets/ sachets)	13.1 (51)
• IM diclofenac	7.7 (30)
• Oral naproxen	12.8 (50)
Antithrombotics	
The demand did not change.	15.9 (62)
The demand increased significantly	84.1 (328)
• Aspirin	79.7 (311)
• Clopidogrel	7.7 (30)
• Warfarin	2.1 (8)
• Heparin	6.7 (26)
• Enoxaparin	31.5 (123)
• Others	7.2 (28)
Anxiolytics and sedatives	
The demand did not change.	69.0 (269)
The demand increased significantly	31.0 (121)

	% (n)
Antidepressants	
The demand did not change.	79.0 (308)
The demand increased significantly	21.0 (82)
Minerals and vitamins	
The demand did not change.	0
The demand increased significantly	100 (390)
• Zinc	98.5 (384)
• Magnesium	14.9 (58)
• Vitamin C	97.7 (381)
• Vitamin D	88.7 (346)
• Vitamin B	16.2 (63)
• Vitamin E	5.6 (22)
• Multivitamins	43.8 (171)
• Iron salts	13.8 (54)
Herbals and supplements	
The demand did not change.	19.5 (76)
The demand increased significantly	80.5 (314)
• Propolis	28.5 (111)
• Omega 3 fatty acids	51.8 (202)
• Immune boosting supplements	46.7 (182)
Cold and cough preparations	
The demand did not change.	15.1 (59)
The demand increased significantly	84.9 (331)
Mucolytics and expectorants	
The demand did not change.	21.8 (85)
The demand increased significantly	78.2 (305)
Antihistamines	
The demand did not change.	33.6 (131)
The demand increased significantly	66.4 (259)

Patterns of non-drug items demand

Among the non-drug items demanded during the COVID-19 pandemic, thermometers reported the highest increase in demand (89%, n=347) followed by oximeters (85.1%, n=322), oxygen generators (68.2%, n=266), antiseptic lozenges (67.9%, n=265), and nebulizers (67.4%, n=263) as shown in Table 3.

Table 3. The pattern of non-drug items demanded during COVID-19 in Jordan as perceived by pharmacists (N=390)

Non-drug items	The demand increased significantly	The demand didn't change	The demand decreased significantly
- Antiseptics lozenges e.g Strepsils®, Halls®, Ricola®	67.9 (265)	30.5 (119)	1.5 (6)
- Normal saline (IV infusion)	43.3 (169)	53.1 (207)	3.6 (14)
- Oximeters	85.1 (332)	12.6 (49)	2.3 (9)
- Thermometers	89.0 (347)	10.8 (42)	0.3 (1)
- Nasal solutions containing normal saline or seawater	64.4 (251)	34.1 (133)	1.5 (6)
- Clove oil	37.7 (147)	58.2 (227)	4.1 (16)
- Menthol rub	52.6 (205)	43.6 (170)	3.8 (15)
- Nebulizers of inhaled drugs	67.4 (263)	31.8 (124)	0.8 (3)
- Oxygen Concentrators/generators	68.2 (266)	29.5 (115)	2.3 (9)

As demonstrated in Table 4, in more than 50% of the interactions with customers, less than half of the pharmacists indicated that their customers provided prescriptions for prescribed drugs.

Table 4: providing a prescription for prescribed drugs

Prescription Drug	Provided prescription in less than 25% of encounters	Provided prescription in 25-49% of encounters	Provided prescription in 50-75% of encounters	Provided prescription in more than 75 % of encounters
Antibiotics	38.1 (138)	44.2 (160)	14.4 (52)	3.3 (12)
Antivirals	32.8 (114)	34.8 (121)	17.5 (61)	14.9 (52)
Analgesics	72.4 (257)	20.3 (72)	6.2 (22)	1.1 (4)
Antithrombotics	38.8 (137)	35.7 (126)	16.1 (57)	9.3 (33)
Anxiolytics or antidepressants	34.4 (118)	24.2 (83)	20.7 (71)	20.7 (71)

Table 5 shows the association of drug demand with the type of pharmacy (independent/chain) and the pharmacy's location in terms of districts. A significant association was seen between the increased demand for antivirals and herbal supplements and chain pharmacies (p-value 0.037 and p-value <0.005, respectively). On the other hand, there was no association between the demand for prescription or non-prescription drugs and the pharmacy's location. Additionally, no significant correlation was found between gender, years of experience, or pharmacy location and drug demand (p>0.05).

Table 5. The pattern of drugs demanded during COVID-19 in Jordan as perceived by pharmacists (N=390)

Drug's category		Area categories and districts cross-tabulated with the drug demand patterns				
		Pharmacy type		***District location***		
		Chain	***Independent***	***Central***	***South***	***North***
Prescription drugs	Increased	97 (97)	97.6 (283)	97.8 (312)	100 (12)	94.9 (56)
1. Antibiotics	Unchanged	3 (3)	2.4 (7)	2.2 (7)	0 (0)	5.1 (3)
	P value	0.721[a]		0.412[a]		
	Increased	75 (75)	63.1 (183)	68 (217)	8 (66.7)	55.9 (33)
2. Antivirals	Unchanged	25 (25)	36.9 (107)	32 (102)	33.3 (4)	44.1 (26)
	P value	*0.037*[b]		0.205[a]		
	Increased	36 (36)	29.3 (85)	32.6 (104)	16.7 (2)	25.4 (15)
3. Anxiolytics and sedatives	Unchanged	64 (64)	70.7 (205)	67.4 (215)	83.3 (10)	74.6 (44)
	P value	0.259[b]		0.375[a]		
	Increased	24 (24)	20 (58)	20.1 (64)	16.7 (2)	27.1 (16)
4. Antidepressants	Unchanged	76 (76)	80 (232)	79.9 (255)	83.3 (10)	72.9 (43)
	P value	0.477[b]		0.420[a]		
	Increased	88 (88)	82.8 (240)	83.7 (267)	83.3 (10)	86.4 (51)
5. Antithrombotic	Unchanged	12 (12)	17.2 (50)	16.3 (52)	16.7 (2)	13.6 (8)
	P value	0.267[b]		0.867[a]		
Nonprescription drugs	Increased	98 (98)	96.6 (280)	97.2 (310)	100 (12)	94.9 (56)
6. Analgesics and antipyretics	Unchanged	2 (2)	3.4 (10)	9 (2.8)	0 (0)	5.1 (3)
	P value	0.738[a]		0.597[a]		
	Increased	93 (93)	76.2 (221)	80.9 (258)	83.3 (10)	78 (46)
7. Herbal supplements	Unchanged	7 (7)	23.8 (69)	19.1 (61)	16.7 (2)	22 (13)
	P value	*<0.005*		0.882[a]		
	Increased	90 (90)	83.1 (241)	84 (268)	100 (12)	86.4 (51)
8. Cold and cough preparations	Unchanged	10 (10)	16.9 (49)	16 (51)	0 (0)	13.6 (8)
	P value	0.107[b]		0.379[a]		
	Increased	79 (79)	77.9 (226)	77.1 (246)	91.7 (11)	81.4 (48)
9. Mucolytics and expectorants	Unchanged	21 (21)	22.1 (64)	22.9 (73)	8.3 (1)	18.6 (11)
	P value	0.889[b]		0.486		
	Increased	65 (65)	66.9 (194)	67.1 (214)	66.7 (8)	62.7 (37)
10. Antihistamines	Unchanged	35 (35)	33.1 (96)	32.9 (105)	33.3 (4)	37.3 (22)
	P value	0.806[b]		0.772		

[a] *p values* were calculated using Fisher's exact test, [b] p values were calculated using Pearson Chi-square test

DISCUSSION

In this study, we evaluated the impact of COVID-19 on the changing demand for medications and non-drug items among Jordanians from the perspectives of pharmacists. During the study period (March to May 2021), Jordan experienced a severe second wave of COVID-19, resulting in the highest daily new cases and deaths since the onset of the pandemic (https://www.worldometers.info/coronavirus/country/jordan/). Given these circumstances, a substantial increase in demand for specific drugs was expected, which was indeed evident in our study. All participants reported an increase in the demand for minerals and vitamins compared to previous years. This rise in demand can be attributed to these supplements being included in Jordan's Ministry of Health's treatment protocol for mild COVID-19 cases (21), allowing self-medication without a prescription. This trend was also noted in other countries such as Kuwait and Nigeria (22,23). The increased demand for vitamins, coupled with the rise in demand for herbal supplements, reflects the public's concerns about enhancing their immunity and protecting themselves from this viral infection.

Nearly 97% of participants indicated a significant increase in the demand for antibiotics, primarily macrolides and fluoroquinolones. Azithromycin was one of the drugs recommended for managing COVID-19 patients during a certain period of the pandemic (24). Similarly, some reports proposed the use of fluoroquinolones as adjunct treatment for COVID-19 (25). This increased demand was likely fueled by information circulating on social media regarding their use in COVID-19 management (26). This demand was boosted by the fact that antibiotics in Jordan can be procured from pharmacies without a prescription (11). Moreover, around 82% of the participants indicated that antibiotics were requested without a prescription in more than 50% of interactions. In Jordan, although regulations prohibit the dispensing of antibiotics without a prescription, these regulations are not strictly enforced (11). This trend of self-medication with antibiotics during COVID-19 was observed in other countries as well (6,17,27,28).

A substantial rise in demand for analgesics and antipyretics (96.9%) was reported, with paracetamol being the most requested drug. This aligns with the common symptom profile of COVID-19, which includes fever and muscle aches. Paracetamol is recommended by the Ministry of Health (21) and the CDC for treating these symptoms as a home remedy for mild cases (29). It was also the most consumed medication during COVID-19 lockdowns in Peru and Yemen (5,17).

Overall, there was a notable increase in demand for drugs used to alleviate COVID-19 symptoms, such as cough and cold preparations and mucolytics and expectorants.

Additionally, a significant increase was observed in the demand for antithrombotic drugs (mainly aspirin and enoxaparin) and, to some extent, anxiolytics and sedatives. It is apparent from Table 4 that the increase in demand for these drugs was for self-medication since, in the majority of encounters, less than 50% of customers provided prescriptions for these drugs. The demand for antithrombotic drugs was likely due to the thromboinflammatory syndrome associated with severe to critical COVID-19 illness (30), which prompted recommendations for their use in specific cases (21). Subsequently, aspirin, being a cheap and over-the-counter drug, was the most demanded in this group. However, it is worth mentioning that health authorities do not recommend the use of these drugs for non-hospitalized patients with COVID-19 to treat venous thromboembolism or arterial thrombosis unless they were indicated for other conditions (31).

Epidemics and pandemics are recognized to heighten the prevalence of psychological disorders driven by stress, anxiety, and fear (32). This concern prompted the WHO and CDC to emphasize the importance of mental health during the COVID-19 pandemic (33,34). In Jordan, these

psychological effects were evident as approximately one-third of our study participants reported an increased demand for anxiolytics and antidepressants. Consistent with our findings, a recent study among healthcare workers in Jordan revealed that 40% of the participants had suffered from severe depression (35). During the pandemic, efforts have primarily focused on treating affected individuals and containing the spread of the disease, with less attention paid to the psychological impact on the population. Based on our findings, it is crucial for health authorities to prioritize mental health during epidemics, providing guidance and support to help individuals maintain their mental well-being.

We also noted a substantial increase in the demand for non-drug items among participants, including thermometers, oximeters, and oxygen generators. The shortage of medical oxygen has been a global issue during the COVID-19 pandemic, with supply falling short of the increased demand (36,37). This problem was particularly acute in poorer countries already grappling with oxygen supply shortages (36,37). It appears that Jordanians sought to acquire oxygen concentrators/generators for home use, contributing to the increased demand for these devices during the study period. However, to prevent the misuse of these concentrators or the unwarranted confidence that they might provide, leading to a false sense of control over a patient's condition at home, it is crucial to educate pharmacists and raise public awareness about the correct use and potential risks associated with these devices. Such education is paramount to ensure that patients seek medical attention when necessary.

We sought to investigate whether changes in drug demand were associated with specific regions within Jordan. The districts were categorized into three regions: north, central, and south, and a statistical analysis was performed among these regions (see Table 5). The central region includes the capital city Amman, which has a higher proportion of the population and wealthier individuals. The results revealed no significant difference, indicating that changes in drug requirements were similar throughout the country, regardless of social, financial, or spatial differences.

Likewise, no significant difference in the change in drug demand was found between chain and independent pharmacies, with the exception of antiviral drugs and herbal supplements. Nevertheless, although a statistical difference was detected in the demand for these two items between chain and independent pharmacies, the demand for these items increased in both types of pharmacies.

CONCLUSION

The COVID-19 pandemic has led to significant changes in the demand for pharmaceutical products, including prescription and non-prescription drugs, supplements, and non-drug items. The public's reaction to the pandemic, in terms of securing medicines and necessary devices to fight and manage the disease, was consistent across different districts. This indicates that social, financial, and spatial differences disappear when lives are threatened by a pandemic. Consequently, pharmacists face increasing pressure to dispense prescription drugs without prescriptions, as well as other supplements and devices. This highlights the importance of preparedness and education for pharmacists to provide correct information and advice to patients. Also, during times of pandemics, health organizations should step up to provide pharmacists with the latest treatments and management protocols to ensure the delivery of accurate information to this frontline health sector. Moreover, health authorities should prioritize mental health during epidemics and provide guidance through various media channels to help individuals maintain their mental well-being. Additionally, attention should be given to the use of various devices for home treatment, with clear advice and guidelines for the public to follow.

Limitations of the study

The questionnaire was distributed through various social media platforms used by pharmacists, including

Facebook, WhatsApp, and Messenger. This could potentially introduce bias since only individuals with internet access or those who are active on these platforms would have the opportunity to participate in the study. Moreover, most participants were young, female, and working in pharmacies located in central Jordan, which could affect the generalizability of the conclusions drawn from the study. Finally, the study was based on the perceptions of pharmacists, which could be subjective and reflect the views of those who responded to the online questionnaire.

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Conflict of Interest

The authors declare no conflict of interest

REFERENCES

1. Ferrer R. COVID-19 Pandemic: the greatest challenge in the history of critical care. *Med Intensiva*. 2020; 44(6):323; doi: 10.1016/J.MEDIN.2020.04.002.
2. Gadhiya KP, Hansrivijit P, Gangireddy M, et al. Clinical characteristics of hospitalised patients with COVID-19 and the impact on mortality: a single-network, retrospective cohort study from Pennsylvania state. *BMJ Open.* 2021; 11(4):e042549; doi: 10.1136/BMJOPEN-2020-042549.
3. WHO. Episode #36 - Safe Care at Home. 2021. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/media-resources/science-in-5/episode-36---safe-care-at-home?gclid=Cj0KCQjwk4yGBhDQARIsACGfAetc5qSaG7lDmV-yVkttTZk6QvWi2OJpPT28PHJnxGbpe0nBfYWtJfAaAnWZEALw_wcB [Last accessed: 6/12/2021].
4. Elayeh E, Aleidi SM, Ya'acoub R, et al. Before and after case reporting: A comparison of the knowledge, attitude and practices of the Jordanian population towards COVID-19. *PLoS One.* 2020; 15(10 October):e0240780; doi: 10.1371/journal.pone.0240780.
5. Quispe-Cañari JF, Fidel-Rosales E, Manrique D, et al. Self-medication practices during the COVID-19 pandemic among the adult population in Peru: A cross-sectional survey. *Saudi Pharm J*. 2021; 29(1):1–11; doi: 10.1016/j.jsps.2020.12.001.
6. Sadio AJ, Gbeasor-Komlanvi FA, Konu RY, et al. Assessment of self-medication practices in the context of the COVID-19 outbreak in Togo. *BMC Public Health*. 2021; 21(1):1–9; doi: 10.1186/s12889-020-10145-1.
7. Mrityunjaya M, Pavithra V, Neelam R, et al. Immune-Boosting, Antioxidant and Anti-inflammatory Food Supplements Targeting Pathogenesis of COVID-19. *Front Immunol.* 2020; 11:2337; doi: 10.3389/FIMMU.2020.570122/BIBTEX.
8. American Pharmacist Association. APhA COVID-19 Resources: Know the Facts_Pharmacists' Guide to Coronavirus | American Pharmacists Association. 2021. Available from: https://www.pharmacist.com/coronavirus.
9. Sousa Pinto G, Hung M, Okoya F, et al. FIP's response to the COVID-19 pandemic: Global pharmacy rises to the challenge. *Res Soc Adm Pharm.* 2021; 17(1):1929; doi: 10.1016/J.SAPHARM.2020.07.004.
10. Iacobucci G. Covid-19: Allow pharmacists to dispense controlled drugs without prescription, urge specialists. n.d.; doi: 10.1136/bmj.m1868.
11. Haddadin RN, Alsous M, Wazaify M, et al. Evaluation of antibiotic dispensing practice in community pharmacies in Jordan: A cross sectional study. *PLoS One.* 2019; 14(4); doi: 10.1371/journal.pone.0216115.

12. Alsous M, Elayeh E, Abdel Jalil M, et al. Evaluation of Self-Medication Practice among Pharmacy Students in Jordan. *Jordan j. pharm. sci.* 2018; 11(1):15–24.
13. Matalqah L, Albals D, Radaideh K, et al. Knowledge, Attitudes and Practice toward Antibiotic Use among Under and PostGraduate Students at Yarmouk University in Jordan: A Descriptive Study. *Jordan j. pharm. sci*. 2022; 15(3):378–389.
14. Elayeh E, Akour A, Haddadin RN. Prevalence and predictors of self-medication drugs to prevent or treat COVID-19: Experience from a Middle Eastern country. *Int J Clin Pract*. 2021; 75(11); doi: 10.1111/IJCP.14860.
15. Al-Azzam S, Mhaidat NM, Banat HA, et al. An Assessment of the Impact of Coronavirus Disease (COVID-19) Pandemic on National Antimicrobial Consumption in Jordan. *Antibiot*. 2021; 10(6):690; doi: 10.3390/ANTIBIOTICS10060690.
16. Brown JD, Vouri SM, Manini TM. Survey-reported medication changes among older adults during the SARS-CoV-2 (COVID-19) pandemic. *Res Soc Adm Pharm*. 2020; doi: 10.1016/j.sapharm.2020.11.005.
17. Saleh EA, Haddadin RN, Saleh B, et al. Changes in drug demand when a pandemic coincides with other outbreaks in a war zone country: a cross-sectional pilot study. *J Pharm Policy Pract*. 2022; 15(1):89; doi: 10.1186/S40545-022-00487-Z.
18. Reuben RC, Danladi MMA, Saleh DA, et al. Knowledge, Attitudes and Practices Towards COVID-19: An Epidemiological Survey in North-Central Nigeria. *J Community Health*. 2021; 46(3):457–470; doi: 10.1007/s10900-020-00881-1.
19. Elayeh E, Aleidi SM, Ya'acoub R, et al. Before and after case reporting: A comparison of the knowledge, attitude and practices of the Jordanian population towards COVID-19. *PLoS One*. 2020; 15(10 October); doi: 10.1371/journal.pone.0240780.
20. Lino M, Di Giuseppe G, Albano L, et al. Parental knowledge, attitudes and behaviours towards influenza A/H1N1 in Italy. *Eur J Public Health*. 2012; 22(4):568–572; doi: 10.1093/EURPUB/CKR115.
21. Jordan-MOH. ا The Protocol of Diagnosis and Treatment of COVID-19 Patients, Issued by the Ministry of Health (Arabic). 2020. Available at: https://corona.moh.gov.jo/ar/MediaCenter/1408 [Last accessed: 12/9/2021].
22. Wegbom AI, Edet CK, Raimi O, et al. Self-Medication Practices and Associated Factors in the Prevention and/or Treatment of COVID-19 Virus: A Population-Based Survey in Nigeria. *Front Public Heal*. 2021; 9:606801; doi: 10.3389/FPUBH.2021.606801/FULL.
23. Ghaith A, Echchad M, AlHaman F, et al. Assessing the Influence of the COVID-19 Pandemic on the Purchasing Intention of Vitamins in Kuwait using the Theory of Planned Behavior. *Jordan j. pharm. sci* 2023; 16(3):565–577.
24. Gautret P, Lagier JC, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents*. 2020; 56(1):105949; doi: 10.1016/J.IJANTIMICAG.2020.105949.
25. Karampela I, Dalamaga M. Could Respiratory Fluoroquinolones, Levofloxacin and Moxifloxacin, Prove to be Beneficial as an Adjunct Treatment in COVID-19? *Arch Med Res*. 2020; 51(7):741–742; doi: 10.1016/J.ARCMED.2020.06.004.
26. Ahmad S, Babar MS, Essar MY, et al. Infodemic, self-medication and stockpiling: a worrying combination. *East Mediterr Health J*. 2021; 27(5):438–440; doi: 10.26719/EMHJ.21.010.
27. Quispe-cañari JF, Fidel-rosales E, Manrique D, et al. Prevalence of self-medication during the COVID-19 pandemic in Peru. SSRN 2020.

28. Nasir M, Chowdhury ASMS, Zahan T. Self-medication during COVID-19 outbreak: a cross sectional online survey in Dhaka city. *Int J Basic Clin Pharmacol*. 2020; 9(9):1325; doi: 10.18203/2319-2003.ijbcp20203522.
29. CDC. Treatments Your Healthcare Provider Might Recommend If You Are Sick | CDC. 2021. Available at: https://www.cdc.gov/coronavirus/2019-ncov/your-health/treatments-for-severe-illness.html [Last accessed: 12/9/2021].
30. Godino C, Scotti A, Maugeri N, et al. Antithrombotic therapy in patients with COVID-19? -Rationale and Evidence-. *Int J Cardiol*. 2021; 324:261; doi: 10.1016/J.IJCARD.2020.09.064.
31. NIH. Antithrombotic Therapy | COVID-19 Treatment Guidelines. 2021. Available at: https://www.covid19treatmentguidelines.nih.gov/therapies/antithrombotic-therapy/ [Last accessed: 12/9/2021].
32. Mak IWC, Chu CM, Pan PC, et al. Long-term psychiatric morbidities among SARS survivors. *Gen Hosp Psychiatry*. 2009; 31(4):318–326; doi: 10.1016/J.GENHOSPPSYCH.2009.03.001.
33. CDC. Coping with Stress. 2021. Available at: https://www.cdc.gov/mentalhealth/stress-coping/cope-with-stress/index.html [Last accessed: 12/9/2021].
34. WHO. #HealthyAtHome - Mental Health. 2021. Available at: https://www.who.int/campaigns/connecting-the-world-to-combat-coronavirus/healthyathome/healthyathome---mental-health [Last accessed: 12/9/2021].
35. Alnazly E, Khraisat OM, Al-Bashaireh AM, et al. Anxiety, depression, stress, fear and social support during COVID-19 pandemic among Jordanian healthcare workers. *PLoS One*. 2021; 16(3):e0247679; doi: 10.1371/JOURNAL.PONE.0247679.
36. Feinmann J. How covid-19 revealed the scandal of medical oxygen supplies worldwide. *BMJ*. 2021; 373; doi: 10.1136/BMJ.N1166.
37. UNICEF. Oxygen Concentrators During COVID-19 | UNICEF China. 2021. Available at: https://www.unicef.cn/en/stories/oxygen-concentrators-during-covid-19 [Last accessed: 12/9/2021].

استكشاف التغيير في طلب المواد الدوائية وغير الدوائية من صيدليات المجتمع خلال جائحة كوفيد-19

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ملخص

خلال جائحة كوفيد 19، واجهت انظمة الرعاية الصحية حول العالم تحديات غير مسبوقة حيث لعب الصيادلة دور مهم على خطوط المواجهة مع المرض. واجه الصيادلة قفزة كبيرة في طلبات المرضى لتأمين الأدوية ومواد غير دوائية لمعالجة او الوقاية من المرض. تهدف هذه الدراسة المستعرضة لتقييم التغييرات في الطلب على بعض االأدوية ومواد غير دوائية في الأردن خلال الفترة من اذار الى حزيران 2022 بناء على إدراك الصيادلة وكذلك للكشف عن العوامل التي تؤثر على هذا الطلب. تم تجهيز إستبانة تستهدف صيادلة المجتمع والتثبت منها وتوزيعها عبر صفحات وسائط التواصل الاجتماعي (واتساب، فيسبوك، ومسنجر) التي تضم الصيادلة. بهذه الدراسة تم جمع بيانات من 390 صيدلاني يعملون بصيدليات المجتمع. بينت النتائج وجود زيادة معتبرة إحصائياً في الطلب على الأدوية التي تحتاج لوصفة طبية مثل المضادات الحيوية (97.4%) ومضادات التخثر (84.1%) ومضادات الفيروسات (66.2%) التي في أغلب الأحيان كانت تطلب دون تقديم وصفة. إزداد الطلب 100% على المواد التي تصرف بدون وصفة مثل المعادن والفيتامينات. كذلك ازداد الطلب على المواد غير الدوائية مثل اجهزة قياس تركيز الاكسجين(85.1%)، وموازين الحرارة (89.0%)، ومولدات الأكسجين (68.2%). التغييرات بطلب الأدوية كانت متشابهة بين محافظات الأردن. على أي حال، تبين وجود ارتباط بين الزيادة في طلب مضادات الفيروسات والمكملات العشبية مع الصيدليات التابعة لسلسلة صيدليات (p-value =0.037 و p-value <0.005على التوالي). في النتيجة، جائحة كوفيد 19 أدت لتغيرات جوهرية في زيادة الطلب على المستحضرات الصيدلانية، مما يشكل ضغط هائل على صيدليات المجتمع. إن تفاعل عامة الناس مع الجائحة لمحاربة وادارة المرض كانت متشابهة عير الأردن بغض النظر عن الفوارق الإجتماعية والمادية والمكانية بين السكان. فهذه الدراسة تسلطالضوء على أهمية تدريب وتحضير الصيادلة لظروف مشابهة ليتمكنوا من تقديم معلومات واستشارات دقيقة للمرضى. بناءً عليه، على السلطات الصحية أن تضمن وصول أحدث بروتوكولات العلاجات وطرق متابعة المرضى للصيادلة وتوفير إرشادات واضحة لعامة الناس عن كيفية إستخدام أجهزة المعالجة المنزلية.

الكلمات الدالة: الأردن، كوفيد-19،الصيدلاني، التداوي الذاتي، صيدليات المجتمع.

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Assessing the Awareness and Attitude Towards COVID-19 Vaccination and Aids Factors among Jordanian People: *A cross-sectional Study*

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ABSTRACT

This study aimed to evaluate the awareness and attitudes toward COVID-19 vaccination among the Jordanian population. A cross-sectional survey was conducted using a validated questionnaire. The awareness and attitudes toward the COVID-19 vaccine were assessed via five-item and seven-item scales, respectively. The survey results were analyzed using SPSS with a chi-square test and multivariable logistic regression. A total of 407 participants were enrolled, with the majority being female (74.9%), under 49 years old (73.2%), holding a bachelor's degree (57.7%), and working in the private sector (46.2%). Results revealed a fairly high level of awareness about the COVID-19 vaccine (51.4%), with no significant association between awareness and demographic characteristics. While 51.4% of the participants perceived the importance of getting the vaccine, only 37.1% agreed that the newly developed vaccine was safe, and 77.4% expressed a preference for natural immunity. The overall attitude towards COVID-19 vaccination appears cautiously optimistic, with 60.2% of respondents scoring above Bloom's 60.0% cutoff point, despite mixed opinions on vaccine safety and necessity. Moreover, attitudes towards the vaccine showed a significant association with participants' age and occupation. Among the age group of 18-29 years old, 47.6% had a positive attitude towards the vaccine, compared to 33.3% in the 30-49 years old group, and 38.5% in the ≥50 years old group. In terms of occupation, 30.7% in the public sector had a positive attitude, compared to 44.1% in the private sector and 48.5% among students. Given the mixed but cautiously optimistic attitudes towards COVID-19 vaccination observed among the Jordanian population, this study underscores the critical importance of targeted educational and communication strategies. Such initiatives should focus on enhancing the perception of vaccine safety and efficacy to improve vaccination acceptance and uptake across different age and occupational groups within Jordan.

Keywords: Awareness, attitude, COVID-19, vaccines.

INTRODUCTION

Vaccination is widely regarded as the most effective intervention to end the pandemic, with an emphasis on achieving herd immunity to limit the spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. However, achieving this goal depends on the population's acceptance of and willingness to receive the vaccine. Vaccine hesitancy, defined as a delay in acceptance or refusal of vaccines despite their availability, is a significant obstacle to achieving herd immunity and is

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influenced by factors such as confidence, complacency, and convenience [2-3].

Coronavirus disease 2019 (COVID-19) is a respiratory infection caused by SARS-CoV-2. Compared to other coronaviruses, SARS-CoV-2 was found to be more infectious, and rapidly spread across the world [4]. COVID-19 has caused millions of deaths worldwide. By February 2023, there were 676,496,910 confirmed cases of COVID-19 globally, with 6,773,736 fatalities, overstretching healthcare systems and prompting a race to develop effective vaccines against SARS-CoV-2 [5].

In response to this, scientists worldwide fast-tracked the development of vaccines using various platforms. By the end of 2020, several COVID-19 vaccines were authorized for emergency use. More than 5.51 billion individuals, or around 71.8% of the world's population, had received a dose of the COVID-19 vaccine by October 2022, with a total of 12,850,970,971 doses administered. According to the World Health Organization (WHO), there were 1,746,997 confirmed cases of COVID-19 in Jordan from January 2020 to November 2022, with 14,122 fatalities. Furthermore, a total of 10,057,975 vaccination doses were administered in Jordan by August 2022 [6], [7].

Vaccines are a cost-effective option for controlling the COVID-19 pandemic, albeit problematic, as morphological variations of SARS-CoV-2 could hinder global efforts for immunization [8]. Vaccine acceptance rates varied widely in a global survey, ranging from less than 55% to more than 90% among participants from 19 nations, with an overall acceptance rate of 71.5%. However, among young Jordanian adults, acceptance of the COVID-19 vaccination was only partial [9]. Furthermore, Jordanians' compliance with non-pharmaceutical intervention (NPI) measures, such as wearing face masks, practicing hand hygiene, and maintaining social distancing, was found to be inadequate [10-11]. Therefore, research on awareness and attitudes towards the vaccine is critical to address and mitigate the negative impact of further COVID-19 infections [12].

Numerous studies have examined the factors that may influence individuals' inclination toward vaccination. There is evidence to suggest that some demographic groups, such as younger individuals, those with lower levels of education, individuals with less income, and ethnic minority communities, exhibit lower rates of vaccination [13]. Another study emphasized the importance of investigating more effective strategies for engaging young people in the process of making medical decisions about their care [3].

RESEARCH OBJECTIVE

Most of the literature on COVID-19 vaccine acceptance and hesitancy has focused on high-income countries, leaving a relative scarcity of research from low- and middle-income countries, including Jordan. Moreover, the rapidly evolving nature of the COVID-19 pandemic and the continuous development and approval of new vaccines necessitate ongoing monitoring and research into public attitudes toward these vaccines. The objective of this research was to assess the awareness and attitudes toward the COVID-19 vaccine and its associated factors in Jordan.

METHODOLOGY

Study Design and Participants

A cross-sectional survey was conducted on adults over the age of 18 using a self-reported online survey. The participants were invited to complete the survey by accessing a link shared through Facebook groups, Facebook Messenger, and WhatsApp over a period spanning September 30, 2022, to December 30, 2022. A study overview was included at the beginning of the questionnaire. Participants completed the survey anonymously to mitigate bias and safeguard confidentiality. They received detailed information on the study's objectives, anticipated duration, and data collection procedures. We determined the sample size considering a significance level (alpha) of 0.05 and a desired statistical power of 80% (1 - Beta). The critical value for a two-tailed

test at the 5% significance level (alpha) was set at 1.96. A total of 307 participants were needed to achieve the target sample size.

Survey instrument

Instrument validity and reliability

The present study's questionnaire was adopted from [14]. The preliminary version of the questionnaire was initially constructed in English and subsequently translated into Arabic. A backward translation from Arabic to English was performed to ensure that the translation maintained the same meaning as the original English version. The instrument was reviewed for content validity by five experts in the field of psychology. Upon receiving their feedback, minor revisions were made to enhance the clarity and precision of the questionnaire items. These included rephrasing unclear or ambiguous questions and making language and grammar corrections. Subsequently, a pilot study with 30 participants confirmed the clarity and feasibility of the instruments. Internal consistency was evaluated using Cronbach's Alpha, obtaining high-reliability coefficients of 90% for attitude and 88% for awareness.

The instrument was structured to align with Bloom's Taxonomy categories for attitude and awareness, with committee cutoff points set at 60.0% for both. The assessment of awareness regarding the COVID-19 vaccine was carried out based on the survey questionnaire modeled after COVID-19 vaccines that had similar structures and content [14].

The awareness questionnaire consisted of five items, with respondents being asked to indicate "yes" or "no" in response to each item. The items were as follows:

- Q1: Do you know about the COVID-19 vaccine?
- Q2: Does the COVID-19 vaccination show effectiveness?
- Q3: Does COVID-19 currently have a therapeutic option?
- Q4: Does COVID-19 currently have a vaccine?
- Q5: What distinguishes the newly developed COVID-19 vaccine from other vaccines?

To assess the attitude level towards the COVID-19 vaccine, respondents were awarded one point for each correct response. The responses were then grouped into two categories: "agree" and "disagree". The survey questions used to evaluate attitudes were:

- Is the newly developed COVID-19 vaccine considered to be safe?
- Is the COVID-19 vaccine considered necessary?"
- Do you plan to get the COVID-19 vaccine?
- Is the COVID-19 vaccine the only treatment available?
- Should the COVID-19 vaccine be distributed equitably?
- Is getting the COVID-19 vaccine necessary?
- Do you prefer natural immunity over the vaccine?

Data Analysis

The Statistical Package for Social Sciences (SPSS - IBM, Chicago, IL, USA) was used to analyze the data. Categorical variables were reported as frequency counts and percentages. A chi-square test was utilized to explore the association between participants' socio-demographic data and their COVID-19 vaccine awareness and attitudes. Furthermore, any variable with a p-value less than 0.20 in the chi-square test was nominated to enter into a multivariable logistic regression model to control for potential confounding. The adjusted odds ratio with corresponding 95% CI was reported to measure the effect size or strength of association. A p-value less than or equal to 0.05 was considered statistically significant.

RESULTS

A total of 407 participants were enrolled in the survey. The majority of the participants were female (305 or 74.9%), under 49 years old (298 or 73.2%), held a bachelor's degree (235 or 57.7%), and were employed in the private sector (188 or 46.2%). Please refer to Table 1 for more details.

Table (1) participants' socio-demographic characteristics

Variable	Category	Frequency	Percentage
Gender	Male	102	25.1
	Female	305	74.9
Age groups	"18-29 years old"	145	35.6
	"30-49 years old"	153	37.6
	"≥50 years old"	109	26.8
Education level	Diploma	58	14.3
	Bachelor	235	57.7
	Higher studies	114	28.0
Occupation	Public sector	153	37.6
	Private sector	188	46.2
	Students	66	16.2

Awareness towards COVID-19 Vaccine:

The distribution of participants on each COVID-19 vaccination awareness item is summarized in Table 2. Regarding the first question, almost all the respondents had heard about the coronavirus vaccine, and more than half, 229 (56.3%), reported that the coronavirus vaccine is effective. A large percentage of the sample, 288 (70.8%), correctly stated that there is currently no effective treatment for coronavirus, and 256 (62.9%) identified that the coronavirus vaccine is different from other vaccines. Based on Bloom's categorization, a total of 209 (51.4%) demonstrated good awareness level towards COVID-19 vaccination.

Table (2) Awareness of COVID-19 Vaccine

Awareness of COVID-19 vaccination	preferred answer	No n(%)	Yes n(%)
1- Have you ever heard of the coronavirus vaccine?	Yes	6(1.5)	401(98.5)
2- Is the coronavirus vaccine effective?	Yes	178(43.7)	229(56.3)
3- Is there an effective treatment for Coronavirus at the current time?	No	288(70.8)	119(29.2)
4-Is there an effective vaccine to prevent coronavirus?	No	257(63.1)	150(36.9)
5-Is the Coronavirus vaccine different from another vaccine	Yes	151(37.1)	256(62.9)
Awareness level based on Bloom's cutoff point (60.0%)	Poor	198	48.6%
	Good	209	51.4%

Attitude towards COVID-19 Vaccine.

The results in Table 3 show that only 151 (37.1%) of participants agreed that the newly discovered vaccine is safe, and 207 (50.9%) of them acknowledged that the COVID-19 vaccine is crucial. Moreover, three quarters of them disagreed that the COVID-19 vaccine is the only solution and agreed that it should be distributed fairly. Furthermore, the results demonstrated that only 209 (51.4%) of participants perceived that it is important to get the vaccine, and nearly three quarters expressed concern about the unforeseen effects of the vaccine and preferred natural immunity. However, according to Bloom's cutoff point, 162 (39.8%) had a positive attitude towards COVID-19 vaccination.

Table (3) Attitude towards COVID-19 Vaccine

Attitude towards COVID-19 vaccination	Disagree	Agree
1-Is the newly discovered vaccine safe?	256(62.9)	151(37.1)
2-Is the COVID-19 vaccine essential for us?	200(49.1)	207(50.9)
3-Is the vaccine the only solution for us?	310(76.2)	97(23.8)
4- If the COVID-19 vaccine should have been distributed fairly!	101(24.8)	306(75.2)
5- Is important to get a vaccine?	198(48.6)	209(51.4)
6- Worried about unforeseen impact?	105(25.8)	302(74.2)
7- Preferred natural immunity rather than the vaccine?	92(22.6)	315(77.4)
Attitude level based on bloom's cutoff point (60.0%)3.93…150	245 162	60.2% 39.8%

As shown in Table 3, only 37.1% of participants agreed that the newly discovered vaccine is safe, suggesting that a majority (62.9%) may have concerns or doubts about the vaccine's safety. Consequently, there is a need for targeted public health messaging and further education to address these concerns. Just over half (50.9%) of participants considered the COVID-19 vaccine essential, indicating a relatively even divide in opinions on the vaccine's importance. The 49.1% who disagree present an opportunity for further intervention and education. The consensus among participants was more significant in response to the claim that the vaccine is the only solution, with 76.2% disagreeing. This suggests that most participants understand the necessity of additional preventative measures, such as wearing masks, maintaining social distance, and practicing hand hygiene. When asked whether the COVID-19 vaccine should be distributed fairly, the majority (75.2%) agreed, indicating broad agreement on the importance of equitable vaccine distribution. Opinions were almost evenly split in terms of whether getting the vaccine is important or whether natural immunity is preferred.

Results of the multiple logistic regression showed that none of the sociodemographic variables were associated with awareness. Regression analysis revealed that those working in the private sector are 1.64 times more likely to have a positive attitude toward the COVID-19 vaccine than those working in the public sector (Table 4).

Table (4) Factors associated with participants' attitude towards COVID-19 Vaccine

Variables	Categories	Attitude n(%)		X^2	Binary logistic regression	
		Negative	Positive	p-value	Adjusted odds ratio (95%C.I)	p-value
Age groups	18-29 years old	76(52.4)	69(47.6)		Ref	
	30-49 years old	102(66.7)	51(33.3)	.041	0.699(0.40-1.22)	.209
	≥50 years old	67(61.5)	42(38.5)		0.872(0.48-1.57)	.647
Occupation	Public sector	106(69.3)	47(30.7)		Ref	
	Private sector	105(55.9)	83(44.1)	.012	1.64(1.01-2.64)	.044
	Students	34(51.5)	32(48.5)		1.70(0.82-3.52)	.155
Gender	Female	187(61.3)	118(38.7)	.427	NA	
	Male	58(56.9)	44(43.1)			
Education level	Diploma	38(65.5)	20(34.5)			
	Bachelor	144(61.3)	91(38.7)	.376		
	Higher studies	63(55.3)	51(44.7)			

DISCUSSION

The COVID-19 pandemic has emphasized the critical role of vaccines in mitigating the spread of the virus, leading to extensive research and approval of various vaccines for global immunization efforts [15]. This study primarily sought to assess the level of awareness and attitudes toward the COVID-19 vaccine among the Jordanian population. In multiple studies exploring public comprehension and receptivity toward the COVID-19 vaccine, varied levels of understanding and sentiments were observed. Our analysis revealed that 51.4% of participants were adequately informed about the vaccine, a finding that aligns with other studies indicating a relatively good level of public awareness. Conversely, a study from Malaysia found that 62% of its participants had insufficient knowledge about the COVID-19 vaccine, even though a promising 64.5% expressed willingness to receive the vaccine, suggesting a positive disposition despite potential misinformation [16]. In Ethiopia, only 40.8% of respondents demonstrated understanding of the COVID-19 vaccines, highlighting a significant knowledge gap and underscoring the need for prompt and effective health education interventions [14]. A separate study focused on Jordan reflected a high inclination toward vaccination, with 72.3% of respondents eager to get vaccinated, indicating a strong pro-vaccination sentiment within the region [17]. Collectively, these findings underscore the need for comprehensive awareness campaigns and targeted educational efforts to reduce information disparities and foster positive perceptions of COVID-19 vaccination globally.

The COVID-19 vaccine is a key strategy to halt the progression of the pandemic, and after rigorous clinical evaluations, several of these vaccines have been authorized in various countries [15]. In Jordan, a strong vaccination campaign is underway, aiming to incorporate the COVID-19 vaccine into the national immunization program [11]. While Jordan has established immunization initiatives, the novel nature of the COVID-19 vaccination campaign prompts questions about its public visibility, interpretation, dissemination, and acceptance [18]. This study sheds light on the current state of vaccine awareness in Jordan and the associated challenges. These findings are crucial in developing tailored awareness and health education campaigns addressing the COVID-19 vaccinations [20].

Our analysis reveals a promising degree of vaccine awareness among Jordanians, with 51.4% of participants demonstrating cognizance—a figure in line with observations from Malaysia [16]. Intriguingly, an individual's educational background played a pivotal role in shaping their awareness about the COVID-19 vaccine, pinpointing the utility of customized educational interventions. However, the diversity in respondent demographics did not substantially influence awareness levels, emphasizing the importance of holistic and inclusive outreach programs [14].

While a praiseworthy 56.3% of participants acknowledged the vaccine's efficacy, this draws parallel to results from Saudi Arabia, emphasizing the incessant need to enlighten both healthcare stakeholders and the broader populace about the vaccine's potency against COVID-19 [20].

Exploring perceptions surrounding the vaccine, the study identified a notable influence of age and professional background on attitudes. Counterintuitively, a sizable portion (39.8%) of younger respondents, aged 18 to 29, exhibited a positive disposition towards the vaccine, defying traditional assumptions [21]. Furthermore, an impressive 51.4% of participants indicated their intent to receive the vaccine. This finding contrasts with some existing literature such as that by [22], suggesting differential trust in various information sources about the vaccine [23-24].

Breaking away from conventional trends, our study found that older cohorts demonstrated a more positive disposition towards the COVID-19 vaccine. This underscores the need for awareness drives tailored

specifically for younger demographics [25].

It is paramount to recognize that vaccine perceptions are shaped by a mosaic of elements—including socioeconomic status, cultural inclinations, healthcare accessibility, and previous experiences with vaccinations. Future research endeavors would benefit from a detailed exploration of these determinants to fully understand vaccine receptivity. Importantly, occupations outside the healthcare sector emerged as significant influencers of vaccination attitudes. Preliminary insights from our research suggest a potential pro-vaccination bias among frontline personnel and those in critical roles. This indicates a need for a more in-depth examination of the interplay between professional domains and vaccine perspectives, especially beyond healthcare settings.

A marked dichotomy between vaccine awareness and attitudes was unveiled in our findings, underscoring the need for refined tactics to reconcile this difference. There was a considerable inclination towards natural immunity over vaccination, highlighting prevailing vaccine apprehensions and indicating the urgency for tailored public health strategies to recalibrate such perceptions. Central to these efforts would be leveraging trusted and credible information sources, emphasizing their crucial role in bolstering public trust and conviction regarding vaccinations [23-24].

When comparing our results with a study conducted in Kuwait by Alibrahim and Awad [26-27], noticeable discrepancies in public awareness concerning the COVID-19 vaccine become apparent. In our analysis, only 37.1% of respondents perceived the newly developed vaccine as devoid of risks, suggesting considerable reservations (62.9%) regarding its safety credentials. Conversely, the Kuwaiti study posited that the main reasons for vaccine reluctance revolved around anxieties linked to potential adverse reactions, perceptions of rushed vaccine development, and skepticism about its protective efficacy, with a significant 57.2% of participants exhibiting a general aversion to vaccines.

Such observations underline the existence of multifaceted reservations and doubts about COVID-19 vaccines across regions, underscoring the pressing need for region-specific strategies to assuage individual apprehensions and bolster collective confidence in the vaccine's safety and effectiveness. Future research exploring additional factors, such as cultural beliefs and socioeconomic status, will contribute to the development of more targeted interventions [28].

Limitations of the study

The cross-sectional design of this study precludes the establishment of a cause-effect relationship. Additionally, the imbalanced gender distribution might affect the accuracy and generalizability of the study findings.

CONCLUSION

In the wake of the COVID-19 pandemic, this research highlights the need to strengthen awareness and attitudes concerning COVID-19 vaccines in order to improve health outcomes. Comprehensive health education campaigns that dispel misinformation, provide accurate information, and emphasize the safety and effectiveness of COVID-19 vaccines are necessary. Tailored communication strategies, accounting for differences in attitudes based on age and occupation, are crucial in building confidence and trust in vaccines. Furthermore, public health campaigns should underline the significance and safety of COVID-19 vaccines to counter the preference for natural immunity.

Conflict of Interest Statement:

The authors declare that there are no conflicts of interest concerning the publication of this work. They have no financial or personal relationships with individuals or organizations that could influence or bias the content of this manuscript inappropriately.

Data Availability Statement:

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Interested researchers can contact the corresponding author to request access to the data. We strongly advocate for the responsible and ethical use of the

data and kindly request that any use of the data in subsequent research or publications be appropriately acknowledged and cited. We are committed to ensuring transparency and reproducibility in our research and aim to make the data available to the scientific community for further analysis and verification of the findings. We appreciate the collaboration and interest of the research community in our work and look forward to contributing to scientific knowledge through the sharing of data.

REFERENCES

1. Anderson RM, Vegvari C, Truscott J, Collyer BS. Challenges in creating herd immunity to SARS-CoV-2 infection by mass vaccination. *The Lancet*. 2020; 21:396(10263):1614-6.
2. MacDonald NE. Vaccine hesitancy: Definition, scope and determinants. *Vaccine*. 2015; 14:33(34):4161-4.
3. Borra SS, Narenthiran CK, Kumar D, Ayilya M. A Comprehensive Review on Efficacy and Adverse Events Associated with Different Covid-19 Vaccines. *Jordan j. pharm. sci*. 2022; 1:15(2):289-304.
4. Niaz K, Nisar MF. Coronavirus Disease-19 (COVID-19): A Perspective of New Scenario (Volume 1) COVID-19: *Epidemiology*.
5. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020; 579(7798):270-3.
6. (WHO Prequalification of Medical Products (IVDs, Medicines, Vaccines and Immunization Devices, Vector Control) - Human Insulin, 2019).
7. Wang C, Chen LY, Lu QB, Cui F. Vaccination with the Inactivated Vaccine (Sinopharm BBIBP-CorV) Ensures Protection against SARS-CoV-2 Related Disease. *Vaccines*. 2022; 9:10(6):920.
8. Fiolet T, Kherabi Y, MacDonald CJ, Ghosn J, Peiffer-Smadja N. Comparing COVID-19 vaccines for their characteristics, efficacy, and effectiveness against SARS-CoV-2 and variants of concern: a narrative review. *Clinical Microbiology and Infection*. 2022; 1:28(2):202-21.
9. Lazarus JV, Ratzan SC, Palayew A, Gostin LO, Larson HJ, Rabin K, Kimball S, El-Mohandes A. A global survey of potential acceptance of a COVID-19 vaccine. *Nature Medicine*. 2021; 27(2):225-8.
10. Al-Qerem W, Jarab AS, Qarqaz R, Hayek MA. Attitudes of a sample of Jordanian young adults toward different available COVID-19 vaccines. Vacunas (English Edition). 2022; 1(23):55-62.
11. El-Elimat T, AbuAlSamen MM, Almomani BA, Al-Sawalha NA, Alali FQ. Acceptance and attitudes toward COVID-19 vaccines: A cross-sectional study from Jordan. *Plos One*. 2021; 23: 16(4):e0250555.
12. Khatatbeh M, Al-Maqableh HO, Albalas S, Al Ajlouni S, A'aqoulah A, Khatatbeh H, Kasasbeh MA, Khatatbeh I, Albalas R, Al-Tammemi AA. Attitudes and commitment toward precautionary measures against COVID-19 amongst the Jordanian population: A large-scale cross-sectional survey. *Frontiers in Public Health*. 2021; 8(9):745149.
13. Al-Taani GM, Muflih S, Alsharedeh R, Altaany Z. Knowledge, Willingness to Pay and Beliefs for Seasonal Influenza Vaccination, A Cross-Sectional Study from Jordan. *Jordan j. pharm. sci*. 2023; 16(4):842-56.
14. Mesesle M. Awareness and attitude towards COVID-19 vaccination and associated factors in Ethiopia: a cross-sectional study. *Infection and Drug Resistance*. 2021; 14:2193-9.
15. Sivan M, Greenhalgh T, Milne R, Delaney B. Are vaccines a potential treatment for long COVID-19? Benefits are possible, but we need more evidence and a mechanism of action. *BMJ-BRITISH MEDICAL JOURNAL*. 2022; 18;377.

16. Mohamed NA, Solehan HM, Mohd Rani MD, Ithnin M, Che Isahak CI. Knowledge, acceptance, and perception on COVID-19 vaccine among Malaysians: A web-based survey. *Plos One*. 2021; 13: 16(8): e0256110.
17. Nusair MB, Arabyat R, Khasawneh R, Al-Azzam S, Nusir AT, Alhayek MY. Assessment of the relationship between COVID-19 risk perception and vaccine acceptance: a cross-sectional study in Jordan. *Human Vaccines & Immunotherapeutics*. 2022; 31: 18(1): 2017734.
18. Lataifeh L, Al-Ani A, Lataifeh I, Ammar K, AlOmary A, Al-Hammouri F, Al-Hussaini M. Knowledge, attitudes, and practices of healthcare workers in Jordan towards the COVID-19 vaccination. *Vaccines*. 2022; 9:10(2):263.
19. Sallam M, Dababseh D, Eid H, Hasan H, Taim D, Al-Mahzoum K, Al-Haidar A, Yaseen A, Ababneh NA, Assaf A, Bakri FG. Low COVID-19 vaccine acceptance is correlated with conspiracy beliefs among university students in Jordan. *International Journal of Environmental Research and Public Health*. 2021; 1:18(5):2407.
20. Ahmed NJ, Alkhawaja FZ, Alrawili AS, Almalki ZS. Public knowledge and attitudes toward COVID-19 Vaccination: A cross-sectional study. *Medical Science*. 2021; 25(108):279-84.
21. Campion FX, Ommen S, Sweet H, Shah N, Rabson B, Dougherty N, Goldsack J, Sylvester P, Jones K, Burgman A, McIntosh N. A COVID-19 telehealth impact study—exploring one year of telehealth experimentation. *Telehealth and Medicine Today*. 2021; 30:6(3).
22. Tolossa T, Wakuma B, Turi E, Mulisa D, Ayala D, Fetensa G, Mengist B, Abera G, Merdassa Atomssa E, Seyoum D, Shibiru T. Attitude of health professionals towards COVID-19 vaccination and associated factors among health professionals, Western Ethiopia: A cross-sectional survey. *PLoS One*. 2022; 9:17(3): e0265061.
23. Verger P, Scronias D, Dauby N, Adedzi KA, Gobert C, Bergeat M, Gagneur A, Dubé E. Attitudes of healthcare workers towards COVID-19 vaccination: a survey in France and French-speaking parts of Belgium and Canada, 2020. *Eurosurveillance*. 2021; 21: 26(3): 2002047.
24. Islam MS, Siddique AB, Akter R, Tasnim R, Sujan MS, Ward PR, Sikder MT. Knowledge, attitudes, and perceptions towards COVID-19 vaccinations: a cross-sectional community survey in Bangladesh. *BMC Public Health*. 2021; 21(1):1-1.
25. Reiter PL, Pennell ML, Katz ML. Acceptability of a COVID-19 vaccine among adults in the United States: How many people would get vaccinated? *Vaccine*. 2020; 29;38(42):6500-7.
26. Alibrahim J, Awad A. COVID-19 vaccine hesitancy among the public in Kuwait: a cross-sectional survey. *International Journal of Environmental Research and Public Health*. 2021; 22:18(16):8836.
27. Al-Qerem W, Jarab AS, Qarqaz R, Hayek MA. Attitudes of a sample of Jordanian young adults toward different available COVID-19 vaccines. Vacunas (English Edition). 2022; 1:23:55-62.
28. Paul E, Steptoe A, Fancourt D. Attitudes towards vaccines and intention to vaccinate against COVID-19: Implications for public health communications. *The Lancet Regional Health–Europe*. 2021; 1:1.

تقييم الوعي والاتجاه نحو التطعيم ضد فيروس كورونا وعوامل المساعدة لدى الشعب الأردني: دراسة مقطعية

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ملخص

هدفت هذه الدراسة إلى تقييم الوعي والموقف تجاه التطعيم ضد فيروس كورونا بين السكان الأردنيين. تم إجراء مسح مقطعي باستخدام استبيان تم التحقق من صحته. تم تقييم الوعي والمواقف تجاه لقاح كوفيد-19 من خلال مقياس مكون من خمسة بنود وسبعة بنود على التوالي. تم تحليل نتائج المسح باستخدام برنامج SPSS مع اختبار مربع كاي والانحدار اللوجستي متعدد المتغيرات. بلغ عدد المشاركين 407 مشاركين، غالبيتهم من الإناث (74.9%)، وأقل من 49 سنة (73.2%)، وحائزات على درجة البكالوريوس (57.7%)، ويعملن في القطاع الخاص (46.2%). أظهرت النتائج وجود مستوى مرتفع إلى حد ما من الوعي حول لقاح كوفيد-19 (51.4%)، مع عدم وجود ارتباط كبير بين الوعي والخصائص الديموغرافية. ومع ذلك، كانت المواقف تجاه اللقاح أكثر تنوعًا. وبينما رأى 51.4% أهمية الحصول على اللقاح، وافق 37.1% فقط على أن اللقاح المطور حديثاً آمن، وأعرب جزء كبير (77.4%) عن تفضيلهم للمناعة الطبيعية. أظهرت المواقف تجاه اللقاح ارتباطًا كبيرًا بعمر المشاركين ومهنتهم. وتؤكد الدراسة على الحاجة إلى التعليم المستمر والتواصل حول فعالية وسلامة لقاحات كوفيد-19، لتعزيز قبول التطعيم وتغطيته بين السكان الأردنيين.

الكلمات الدالة: التوجهات، المواقف، كوفيد-19، اللقاحات.

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Optimizing Drug Delivery Vehicle with Multi-Criteria Decision Making (MCDM) - Based Excipient Selection

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ABSTRACT

Excipients are used in drug delivery systems as a means of effectively delivering drugs to their target site. Multi-criteria decision-making (MCDM) methods are tools for decision-making that consider multidimensional factors. Such methods are a comparative technology used in medicine that combines individual criteria into the total assessment of selected alternatives. This study aims to enhance the solubility and bioavailability of drugs through the application of MCDM-based excipient selection. By incorporating the Preference Ranking Organization Method for Enrichment Evaluations (PROMETHEE), various excipients can be evaluated and ranked based on their suitability for specific applications, considering parameters related to drug solubility and bioavailability. The results highlight the potential of cyclodextrins (net flow: 0.0023) and Eudragit polymers (net flow: 0.0016) as preferred options for drug carriers, while Poloxamer 188 (P188) (net flow: -0.0030) is identified as the least preferred option. This study demonstrates the effectiveness of the PROMETHEE method in improving the performance of poorly soluble and bioavailable drugs, ultimately contributing to the development of new drug delivery systems. The findings have significant implications for therapeutic outcomes in the treatment of diseases.

Keywords: Multi-criteria decision making (MCDM), Drug Carrier excipient, Solubility, Bioavailability.

1.0 INTRODUCTION

The selection of suitable drug carrier excipients in drug delivery systems plays a crucial role in enhancing the solubility and bioavailability of drugs (1). BCS (as seen in Fig 1) is a scientific classification system used for grouping drug products according to their water solution solubility and intestinal permeability. Excipients play a significant role in improving the solubility and permeability of desired drugs. Using several enhancing techniques, drug carrier polymers such as Eudragit, HPMC, Soluplus, HPC, PVP, and many others have proven to improve the apparent solubility and bioavailability of class II and IV drug compounds. Multi-criteria Decision-Making (MCDM) methods provide a robust framework for decision-making processes, considering multiple factors and criteria in the evaluation of selected alternatives. Several authors, such as Alaa Aziz and Fraj Abudayah, have explored the opportunity of drug delivery for treating diseases (2). In the pharmaceutical field, MCDM-based approaches have shown promise in streamlining the excipient selection process, reducing screening time, and optimizing drug delivery vehicles for improved therapeutic outcomes. The motivation behind this study is to leverage the potential advantages of MCDM in pharmaceutics and drug delivery technologies. By employing the Preference Ranking Organization Method for Enrichment Evaluations (PROMETHEE), this research aims to identify and rank various excipients based on their suitability for enhancing drug solubility and bioavailability. The application of fuzzy logic in PROMETHEE facilitates handling complex and uncertain decision-making scenarios, making it well-suited for

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pharmaceutical applications where several criteria need to be considered (2). While excipient selection is a critical aspect of drug development, limited research has focused on the potential advantages of Multi-Criteria Decision-Making (MCDM) methods in this domain. Traditional approaches to excipient selection often involve time-consuming and resource-intensive experimental screenings. This research novelly employs the MCDM-based fuzzy Preference Ranking Organization Method for Enrichment Evaluation (PROMETHEE) for excipient selection, providing a systematic and efficient means of identifying optimal drug carriers.

The proposed methodology integrates the fuzzy sets approach, which allows the inclusion of expert knowledge and linguistic variables in the decision-making process. This enables a more realistic representation of human judgment, making decision outcomes more relevant and applicable to real-world scenarios. This study uniquely combines the benefits of both MCDM and fuzzy sets to optimize the selection of drug delivery vehicles using fuzzy PROMETHEE.

The primary contribution of this study lies in demonstrating the effectiveness of the fuzzy PROMETHEE method for selecting drug carrier excipients to enhance drug solubility and bioavailability. This research provides a comprehensive evaluation of various excipients based on multiple criteria. To the best of our knowledge, this is the first study to innovatively use the fuzzy PROMETHEE method to evaluate drug carrier excipients. No other study in existing literature has compared drug carrier excipients using this methodology.

This research offers pharmaceutical scientists and decision-makers a valuable tool for informed decision-making in drug delivery system design. The potential advantages of MCDM-based excipient selection include reduced screening time and efforts, cost-effectiveness, and improved therapeutic outcomes. Additionally, the application of fuzzy logic in PROMETHEE allows for handling uncertainties and ambiguities often encountered in pharmaceutical decision-making processes. The findings of this study hold significant implications for drug development and therapeutic advancements in the treatment of diseases, including oncology and rare genetic conditions.

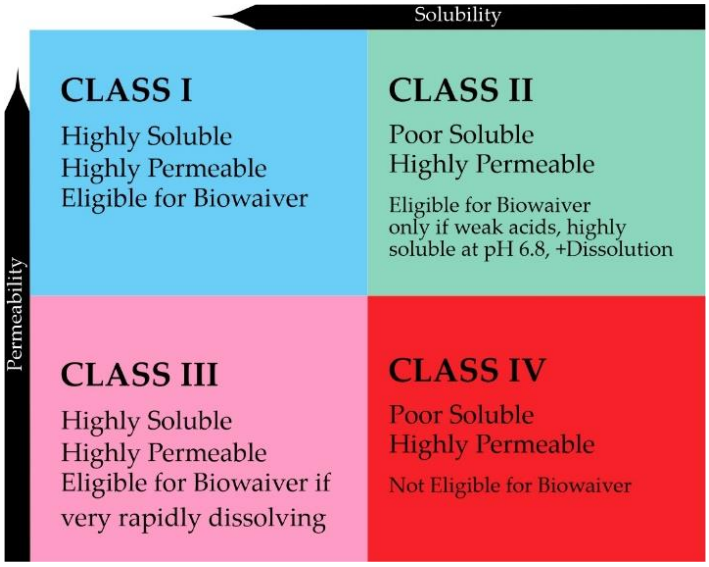

Figure 1: BCS Classification of drug (Author, 2023)

This current study focuses on frequently used drug carrier excipients over the past 10 years. The selected excipients have varied properties, disparate chemical compositions, and diverse means of drug delivery.

1.1 Cyclodextrins

Cyclodextrins are ring-shaped oligosaccharides naturally produced when bacteria act upon cellulose. They are composed of larger glucose molecules joined together by α-1,4 glycosidic bonds. While some types may possess as many as 6 units of glucose, others bear 7 to 8 units (β-, and γ- cyclodextrins, respectively) (Fig 2a). Cyclodextrins can encapsulate drugs and release them at the site of absorption (Fig 2b), thereby enhancing the solubility and bioavailability of the drug. Their applications include the crosslinking of water-soluble cyclodextrin with hyaluronic acid for targeted drug delivery for wound treatment (3).

Figure 2a: Chemical structure of alpha, beta, and gamma cyclodextrins (Nikitenko et all, 2013)

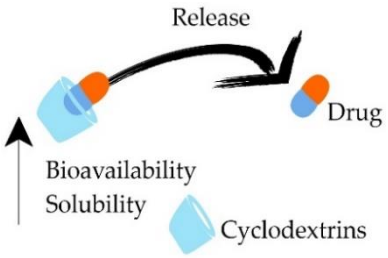

Figure 2b: Cyclodextrins as a drug carrier (Author, 2023)

1.2 Soluplus

The application of these polymers in enhancing technologies such as amorphous solid dispersions aim to maintain the supersaturation of the drug molecules by forming micelles around the poorly soluble drugs thereby inhibiting recrystallization of the drug. The stability of enhanced drugs by amorphous solid dispersions by micelles continues to be a concern as the micelle's formations do not stay around the drugs indefinitely.

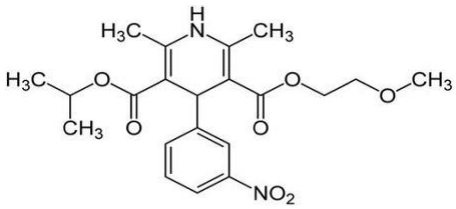

Figure 3a: Chemical Structure of Soluplus (PCL-PVAc-PEG) (Alsheyyab et all, 2019)

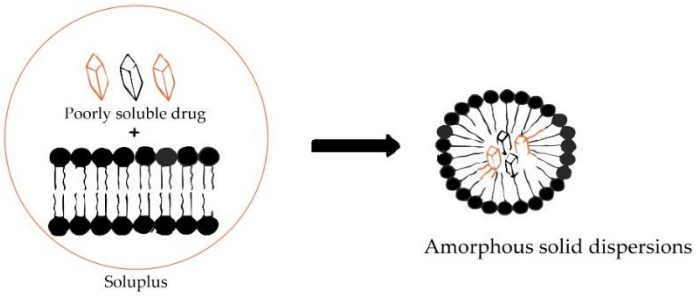

Figure 3b: Soluplus Polymeric Micells (Author, 2023)

1.3 Hydroxypropyl methylcellulose (HPMC)

HPMC, or Hydroxypropyl Methylcellulose, is a hydrophilic polymer used in the production of orally based drug delivery systems. It possesses significant properties such as high swelling capability and delayed release characteristics. Hydroxypropyl methylcellulose is a semi-synthetic, inert, viscoelastic polymer. Several formulations based on HPMC have been synthesized and evaluated for use as gastro retentive drug delivery systems, including tablets, capsules, pellets, and microparticles.

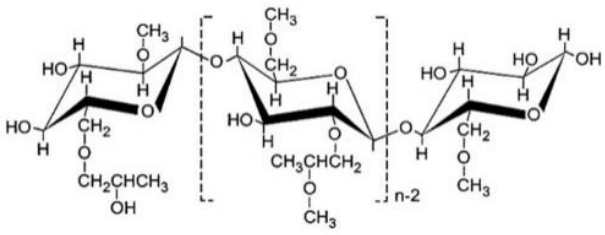

Figure 4: Chemical structure of Hydroxypropyl methylcellulose (Azad et all, 2019)

1.4 Poly (vinyl pyrrolidone) PVP

Polyvinylpyrrolidone (PVP), also known as Povidone, is a water-soluble material obtained through the polymerization of the monomer N-vinyl-pyrrolidone (Fig 5). PVP can encapsulate both hydrophilic and lipophilic drugs. Its inert and non-toxic properties, temperature resistance, and stability across various pH ranges contribute to its selection as a choice ingredient in drug delivery systems.

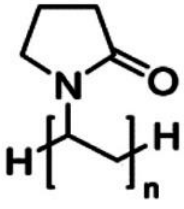

Figure 5: Chemical structure of Polyvinylpyrrolidone (Sigma Aldrich, 2023)

1.5 Poloxamers

Poloxamers are used as carriers to improve the solubilization and stability of compounds. They can form micelles and act as nanocarriers of drugs to the site of action. The surfactant property of poloxamer allows it to be inserted into lipid monolayers, thereby promoting the solubility and bioavailability of drugs.

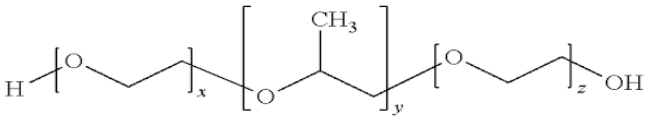

Figure 6: Chemical structure of Poloxamer (Specialized RX, 2023)

1.6 Eudragit Polymers

Eudragit polymers offer a wide range of polymers with enhancing characteristics to optimize the bioavailability, stability, and drug load of the final product. Eudragit is prepared by the polymerization of acrylic and methacrylic acids, or their esters, as seen in Figure 7.

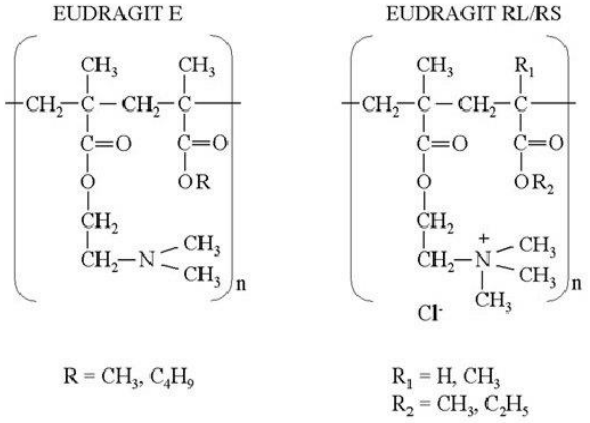

Figure 7: Chemical Structure of Eudragit (Nguyen et all, 2006)

2.0 Multi Criteria Decision Making (MCDM)

The diversity of Multi-Criteria Decision-Making (MCDM) methods ensures that decision-makers have a wide range of tools at their disposal to handle various decision-making challenges effectively. In this study, the fuzzy Preference Ranking Organization Method for Enrichment Evaluations (PROMETHEE) method has been deployed to evaluate the available alternatives based on selected and weighted parameters. The use of MCDM has gradually gained traction with agencies for healthcare technological advancement, and its incorporation is progressively occurring across Europe. It's vital to carefully select the MCDM method that aligns with the complexity of the problem, data availability, and the level of ambiguity present. It's important to note that no single MCDM method is innately more valuable than the others, as their significance depends on the specific problem context and the decision-maker's preferences and priorities.

2.1 Fuzzy PROMETHEE

The PROMETHEE method is one of the most recent Multi-Criteria Decision Analysis (MCDA) methods. It was developed by Brans (1982) and later expanded by Vincke and Brans (1985). PROMETHEE is a tool used for sorting a number of closely related alternatives from which selections are ranked and chosen based on clearly stated criteria. Fuzzy PROMETHEE is an extension of the traditional PROMETHEE method that incorporates fuzzy logic to handle uncertainties and linguistic variables. It allows for more flexible and realistic decision-making, making it suitable for situations with imprecise or ambiguous data (4).

Several studies have utilized the fuzzy PROMETHEE technique in the field of medicine. For example, a study by Ozsahin (2022) (5) used the fuzzy PROMETHEE technique for the selection of radiopharmaceutical tau PET tracers to significantly improve the diagnosis and treatment accuracy of neurodegenerative diseases for targeted and personalized imaging based on precision. Another study by Uzun (2023) (6) used fuzzy PROMETHEE to evaluate machine learning models used for the real-time detection of brain tumors, facilitating early diagnosis and effective management of brain cancer. Similarly, Onakpojeruo (2022) (7) compared the treatment alternatives for spinal cord tumors using the same MCDM technique, contributing to the existing body of knowledge that prioritizes the treatment approaches for spinal cord tumors. These studies and many others demonstrate the effectiveness of MCDM methods in decision-making, particularly in the healthcare field.

3.0 METHODOLOGY

Before proceeding with the implementation of fuzzy PROMETHEE, we simplified the process by assigning relative weights to each of the criteria, as shown in Table 1. The weighted average scores of all the criteria used to rank the various options are displayed in Table 2. Additionally, we used the Yager index to remove any ambiguity that may have resulted from the data used. The Yager index is a promising candidate for defuzzification, as it comprehensively covers all potential set points, making it an ideal choice for this process. During the analysis process, we utilized a Gaussian preference function to make decisions regarding the PROMETHEE strategy.

Putting a numerical value on each criterion is a common approach to prioritize different criteria and draw attention to their relative importance. When using the fuzzy PROMETHEE method, the order in which various options are ranked depends on distinct factors. These factors include the criteria used, the considered alternatives, the weights assigned to different criteria, and the preset preferences. To correctly assign criteria, it is necessary to conduct a thorough analysis of the relevant literature and consult field experts.

Table 1. Linguistic variables and assigned fuzzy numbers with their corresponding priority weight.

Linguistic scale	Fuzzy Number/criteria weight	Rating of criteria
Very high (VH)	(0.75, 1.00, 1.00)	Solubility, Bioavailability
High (H)	(0.50, 0.75, 1.00)	side effect
Moderate (M)	(0.25, 0.50, 0.75)	Food effect, Stability of Drug Product, Drug Load, Dosage Form, Manufacturing Cost.
Low (L)	(0, 0.25, 0.50)	Ease of Manufacturing, Formulation Technology Selection
Very low (VL)	(0, 0, 0.25)	Sustained or Delayed Release Modification of Drug

Table 2. Drug carrier parameters evaluated with visual PROMETHEE values

criteria/alternatives	Aim	Weight	Cyclodextrins	Soluplus (PCL-PVAc-PEG)	Hydroxypropyl methylcellulose (HPMC)	Poly (vinyl pyrrolidone) (PVP)	Poloxamer	Eudragit Polymers
Solubility	Max	VH (0.92)	H	VH	M	H	H	VH
Bioavailability	Max	VH (0.92)	M	H	M	H	H	VH
Food effect	Min	M (0.50)	L	M	L	M	M	M
Stability of Drug Product	Max	M (0.50)	L	L	H	M	L	M
Sustained or Delayed Release Modification of Drug	Max	VL (0.08)	H	L	VH	L	L	VH
Drug Load	Max	M (0.50)	H	M	M	M	M	M
Dosage Form	Max	M (0.50)	VH	H	L	M	L	M
Side Effect	Min	H (0.75)	L	M	M	M	M	M
Manufacturing Cost	MIn	M (0.50)	L	M	H	L	M	M
Ease of Manufacturing	Max	L (0.25)	H	VH	H	H	L	VH
Formulation Technology Selection	Max	L (0.25)	VH	VH	L	H	L	H

3. RESULT AND DISCUSSIONS

In this study, drug carrier excipients, including cyclodextrins, soluplus (PCL-PVAc-PEG), hydroxypropyl methylcellulose (HPMC), poly(vinyl pyrrolidone) (PVP), poloxamer, and Eudragit polymers, were evaluated using a combination of fuzzy and PROMETHEE methods. The evaluation utilized criteria such as enhancements in solubility, bioavailability, the food effect, drug stability, drug load, manufacturing cost, side effects, manufacturing ease, ability to sustain or delay drug release, swallowability, and taste masking. Relative weights were assigned to each criterion, and their weighted average scores were used to rank the various options. The Yager index was employed to clarify any potential confusion caused by the data.

The drug carriers and their excipients were ranked according to their net flow scores, which took into account both the positive and negative flows between various criteria. The PROMETHEE method used in the study generates an important output known as the NetFlow score. This score represents the difference between the positive "outranking" flow and the negative "outranking" flow for each evaluated option—essentially, it measures the degree to which an option outperforms the other options.

Within this investigation's context, the NetFlow score provides a quantitative indication of the overall ranking of various drug carriers and excipients. Alternatives with higher positive NetFlow scores are considered the best choices as they exhibit a higher degree of preference compared to other options within the set. Conversely, options with negative NetFlow scores are viewed as less desirable alternatives due to their lower ranking. Therefore, NetFlow scores' significance lies in their ability to establish a distinct ranking of drug carriers and excipients, based on each option's overall preference concerning the evaluation criteria.

The findings from the investigation, as shown in Table 3, indicate that cyclodextrins ranked first with a net flow score of 0.0023, followed by Eudragit polymers with a net flow score of 0.0016. Soluplus (PCL-PVAc-PEG) came in third with a net flow score of 0.0011, and Polyvinylpyrrolidone (PVP) was fourth with a net flow score of 0.0005. The Hydroxypropyl Methylcellulose (HPMC) drug carrier excipient ranked fifth, with a negative net flow score of -0.0025, indicating it held a lower rank than the other excipients used in drug carriers. Poloxamer, with a net flow score of -0.0030, came in sixth and was considered the least desirable drug carrier excipient according to the criteria used in the analysis.

It's important to underscore the significance of a drug's property in its carrier selection. We used the graphical PROMETHEE rainbow to assess how well each potential drug carrier aligned with the criteria, showcasing the positive and negative attributes. Based on the considered criteria and their respective weights, Figure 8 shows that cyclodextrins are the most advantageous and preferred drug carrier excipient, while poloxamer ranked last.

3.1. Further Discussions

This study used a unique methodology that integrated fuzzy logic and the PROMETHEE method to evaluate and rank drug carrier excipients based on their ability to improve medication solubility and bioavailability. The careful selection of suitable drug carriers is a crucial component in the development of effective drug delivery systems. Various factors, including solubility, bioavailability, food effect, medication stability, and production cost, significantly impact drug carrier effectiveness. However, offering a qualitative assessment of the optimal excipient selection lacks methodological rigor. The current study seeks to overcome this limitation by providing a quantitative and systematic evaluation of diverse drug delivery systems.

To ensure clarity, we assigned relative weights to each criterion according to their respective importance. The weights were derived using linguistic variables and fuzzy numbers, which served as a medium to convert expert opinions from qualitative to quantitative forms. The selected criteria were pivotal in assessing the excipients.

The PROMETHEE approach was used for a thorough evaluation of the drug carriers, in line with the determined criteria. As explained earlier, the net flow scores acquired from the PROMETHEE analysis represent the collective preference of each drug carrier, considering both positive and negative flows. Positive NetFlow scores indicate a greater level of preference compared to other options within the group, while negative NetFlow scores suggest lesser desirability.

When examining the results, it was observed that cyclodextrins demonstrated the most advantageous characteristics, as evidenced by achieving the highest NetFlow score (0.0023). In contrast, Poloxamer obtained the lowest NetFlow score (-0.0030), indicating its status as the least preferred excipient. The obtained results establish a definitive hierarchy among the drug carriers, as determined by their overall preference concerning the defined parameters.

This research's novelty lies in its innovative application of Multi-Criteria Decision Making (MCDM) methodologies, namely fuzzy logic and PROMETHEE, within the field of medicine, specifically for excipient selection. Traditional approaches often depend on subjective assessments, lacking a rigorous scientific foundation. Our approach addresses this constraint by quantifying qualitative factors and providing a structured, scientifically determined evaluation of drug carriers, utilizing a comprehensive set of criteria.

Table 3: PROMETHEE results for drug carriers excipients

Rank	Drug carriers	Outranking NetFlow	Phi+ NetFlow	Phi- NetFlow
1	Cyclodextrins	0,0023	0,0035	0,0012
2	Eudragit Polymers	0,0016	0,0023	0,0007
3	Soluplus (PCL-PVAc-PEG)	0,0011	0,0021	0,0010
4	Poly(vinyl pyrrolidone) (PVP)	0,0005	0,0014	0,0010
5	Hydroxypropyl methylcellulose (HPMC)	-0,0025	0,0014	0,0040
6	Poloxamer	-0,0030	0,0004	0,0034

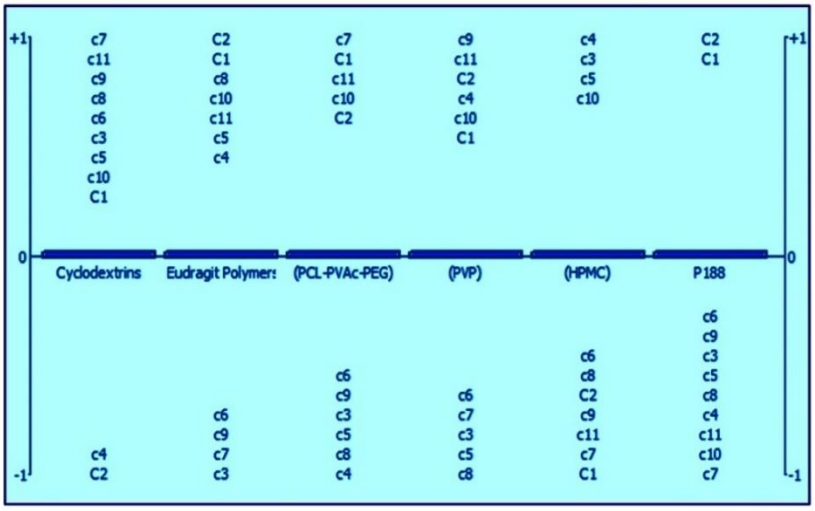

Figure 8: PROMETHEE rainbow of positive and negative aspects of the drug carriers

NB: C1= Solubility, c2 = Bioavailability, c3 = Food effect, c4 = Stability of Drug Product, c5 = Sustained or Delayed Release Modification of Drug, c6 = Drug Load, c7 = Dosage Form, c8 = Side Effect, c9 = Manufacturing Cost, c10 = Ease of Manufacturing, c11 = Formulation Technology Selection

4. CONCLUSION

Solubility and bioavailability continue to present major challenges in drug delivery. To increase a drug's efficacy, a drug carrier excipient is often used. However, the selection of this carrier is based on a multitude of factors, which can make choosing the optimal carrier quite difficult. In this study, we evaluated the most frequently used excipients in the pharmaceutical industry and ranked them in order according to their PROMETHEE values. This straightforward yet efficient technique allows for swift screening of suitable excipients among a vast array of choices. This time-saving method can also be extended to a more extensive selection of excipients, yielding similar results.

While we acknowledge that every drug molecule is unique and may respond differently to drug carriers, the MCDM tool serves as a robust guide in screening and reducing the need for multiple experimental runs, thereby saving time, money, and resources. The results from the analysis show that cyclodextrins are the preferred drug carrier excipients, followed by Eudragit polymers and then Soluplus, while Poloxamer is the least preferred alternative based on the evaluation criteria.

Future research should focus on validating the results through experimental trials, investigating how the chosen excipients perform concerning actual drug delivery and bioavailability enhancement. Additionally, integrating more complex criteria and refining the linguistic variables could further improve the accuracy and applicability of the method.

The findings of this study hold significant implications for drug development and therapeutic advancements in pharmaceutical research and development. Researchers, pharmaceutical scientists, and decision-makers can leverag these findings to make informed choices in drug delivery system design, thus positively impacting the field.

Conflict of Interest

The authors declare no conflict of interest.

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REFERENCES

1. Chavda H.V, Patel C.N and Anand I.S. Biopharmaceutics Classification System. *Sys Rev Pharm*. 2010;1.
2. Alaa Aziz, Fraj Abudayah. Implication of Nanotechnology for Pulmonary Delivery of Docetaxel. *Jordan j. pharm. sci*. 2023;16(2), Supplement 1, 470.
3. Fedaa Adaileh. Crosslinking of Water-Soluble Cyclodextrin with Hyaluronic Acid for Targeted Drug Delivery. *Jordan j. pharm. sci*. 2023;16(2), Supplement 1, 458.
4. Zimmermann, H., J. Description, and optimization of fuzzy systems. *Inter-nat. J. General Systems*. 1976;209-215.
5. Ozsahin, I. Identifying a personalized anesthetic with fuzzy PROMETHEE. *Healthcare Informatics Research*. 2020; 26(3):201-211.
6. Uzun Ozsahin, D., Onakpojeruo, E. P., Uzun, B., Mustapha, M. T., & Ozsahin, I. Mathematical Assessment of Machine Learning Models Used for Brain Tumor Diagnosis. *Diagnostics*. 2023; 13(4): 618.
7. Onakpojeruo, E. P., Uzun, B., Ozsahin, I., & Ozsahin, D. U. Evaluation of the Treatment Alternatives for Spinal Cord Tumors Using Analytical Evaluation Models; 2011.
8. Nikitenko, Natalia & Prassolov, Vladimir. Non-Viral Delivery and Therapeutic Application of Small Interfering RNAs. *Acta naturae*. 2013;5:35-53. 10.32607/20758251-2013-5-3-35-53.
9. Alsheyyab, Rawda & Obaidat, Rana & Al Tall, Yara & Abuhuwaij, Rana & Ghanma, Rand & Ailabouni, Anoud & Mashaqbeh, Hadeia & Al-Haj, Shayma. Solubility enhancement of nimodipine through preparation of Soluplus ® dispersions ARTICLE INFO. *Journal of Applied Pharmaceutical Science*. 2019.9:30-037. 10.7324/JAPS.2019.90905.
10. Azad, Obyedul & Kang, Wie & Lim, Jung & Park, Cheol. Bio- Fortification of Angelica gigas Nakai Nano-Powder Using Bio-Polymer by Hot Melt Extrusion to Enhance the Bioaccessibility and Functionality of Nutraceutical Compounds. *Pharmaceuticals*. 2019; 13. 3. 10.3390/ph13010003.
11. Polyvinylpyrrolidone Image accessed from https://www.sigmaaldrich.com/US/en/product/sial/81440 [accessed 1 Oct 2023]
12. Poloxamer Image accessed from https://specializedrx.com/products/poloxamer-407-nf-micronized-pluronic-f-127 [accessed 1 Oct 2023]
13. Nguyen, Cung & Konan-Kouakou, Yvette & Allémann, Eric & Doelker, Eric & Quintanar, David & Fessi, Hatem & Gurny, Robert. Preparation of surfactant-free nanoparticles of methacrylic acid copolymers used for film coating. *AAPS PharmSciTech*. 2006;7:63. 10.1208/pt070363.
14. Baki, G., Bajdik, J. And Pintye Hodi, K. Evaluation of powder mixtures and hydrophilic gastroretentive drug delivery systems containing zinc acetate and sodium bicarbonate. *J. Pharm. Biomed*. Anal; 2011;54(4): 711-716.
15. Bartlett and Parelukar, Breast cancers are rare diseases—and must be treated as such. *npj Breast Cancer*. 2017;3:11; doi:10.1038/s41523-017-0013-y.
16. Behzadian, M., Kazemzadeh, R.B., Albadvi, A., Aghdasi, M. PROMETHEE: A comprehensive literature review on methodologies and applications. *European Journal of Operational Research*. 2010; 200:198–215.
17. Boldhane, S. P. And Kuchekar, B. S.Development and optimization of metoprolol succinate gastroretentive drug delivery system. *Acta. Pharm*. 2010;60(4):415-425.
18. Dong, B., Lim, L. M. And Hadinoto, K. Enhancing the physical stability and supersaturation generation of amorphous drug-polyelectrolyte nanoparticle complex via incorporation of crystallization inhibitor at the nanoparticle formation step: a case of HPMC versus PVP, Eur. *J. Pharmaceut. Sci*. 2019;138:105035, https://doi.org/10.1016/j.ejps.2019.105035;
19. Dorozynski, P., Kulinowski, P., Mendyk, A., Jachowicz, R. Gastrorentetive drug delivery systems with L-dopa based on carrageenans and hydroxypropylmethylcellulose. *Int. J. Pharm*. 2011; 404: 169-175.
20. Duwa, B., Onakpojeruo, E. P., Uzun, B., Ozsahin, I., & Ozsahin, D. U. Comparative Evaluation of 3D Filaments, Used in Additive Manufacturing of Biomedical Tools; Using Fuzzy Promethee. 2022

21. Fitch C, Keim KS. Position of the Academy of Nutrition and Dietetics: use of nutritive and nonnutritive sweeteners. *J Acad Nutr Diet*. 2012; 112(5):739–758.
22. Gaikwad, D., Shewale, R., Patil, V., Mali, D., Gaikwad, U. And Jadhav, N. Enhancement in in vitro anti-angiogenesis activity and cytotoxicity in lung cancer cell by pectin-PVP based curcumin particulates, *Int. J. Biol. Macromol*. 104:656–664, https://doi.org/10.1016/j.ijbiomac . 2017; 2017.05.170;.
23. Gajbhiye, V., Escalante, L., Chen, G., Laperle, A. And Zheng, Q. Drug-loaded nanoparticles induce gene expression in human pluripotent stem cell derivatives. *Nanoscale*. 2014;6:521-531.
24. Giri, T.K., Saurabh Verma, S., Ajazuddin, Alexander, A., Badwaik, H. and Tripathi, D.K. Prospective and New Findings of Hydroxypropyl Methylcellulose (HPMC) as a Potential Carrier for Gastrorententive Drug Delivery Systems. *Drug Delivery Letters*. 2012;2:98-107.
25. Gottwald, S. On the existence of solutions of systems of fuzzy equations. *Fuzzy Sets and Systems.* 1984;12:301-302.
26. Gu, J. H., Ge, J.B., Li M., Xu, H. D., Wu, F. And Qin, Z. H. Poloxamer 188 protects neurons against ischemia/reperfusion injury through preserving integrity of cell membranes and blood brain barrier. *PLoS One*. 2013;16:8(4): e61641. doi: 10.1371/journal.pone.0061641.
27. Hao, Y., Shih, H., Munoz, Z., Kemp, A., Lin, C. C. Visible light cured thiol-vinyl hydrogels with tunable degradation for 3D cell culture. *Acta Biomater*. 2014;10: 104-114.
28. Hascicek, C. Rossi, A. Colombo, P. Massimo, G. Strusi, O.L. Colombo, G. Colombo, G. Assemblage of drug release modules: effect of module shape and position in the assembled systems on floating behavior and release rate. *Eur. J. Pharm. Biopharm*. 2011; 77(1):116-121.
29. Hu, L.D. Xing, Q.B. Shang, C. Liu, W. Liu, C. Luo, Z.L. Xu, H.X. Preparation of rosiglitazone maleate sustained-release floating microspheres for improved bioavailability. *Pharmazie*. 2010;65(7):477-480.
30. Hughey, J. R., Kean, J. M., Miller, D. A., Brough, C. And McGinity, J. W. Preparation and characterization of fusion processed solid dispersions containing a viscous thermally labile polymeric carrier, *Int. J. Pharm*. 2012; 438:11-19.
31. Koczkur, K. M., Mourdikoudis, S., Polavarapu, L., Skrabalak, S. E., Koczkur, K. M., Mourdikoudis, S., Polavarapu, L., Skrabalak, P. Pvp, R.S. Chem, Polyvinylpyrrolidone (PVP) in nanoparticle synthesis, R. Soc. *Chem*. 2015;44:17883–17905.
32. Li, B., He, M., Li, W., Luo, W., Guo, Y., Li, Y., Zang, C., Wang, B., Li, F., Li, S. and Ji, P. Dissolution and pharmacokinetics of baicalin–polyvinylpyrrolidone coprecipitate. *J. Pharm. Pharmacol*. 2013; 65:1670-1678.
33. Linkov, I. And Moberg, E. Multi-Criteria Decision Analysis: Environmental Applications and Case Studies. Boca Raton, FL: CRC Press, Taylor & FrancisGroup. 2011
34. McKinnon, D. D., Brown, T. E., Kyburz, K. A., Kiyotake, E. And Anseth, K. S. Design and characterization of a synthetically accessible, photodegradable hydrogel for user-directed formation of neural networks. *Biomacromolecules*. 2014; 15:2808-2816.
35. Miller, D. A., DeNunzio, J. C., Hughey, J. R., Williams III, R. O. and McGinity, J. W. Kinetisol: A new processing paradigm for amorphous solid dispersion system. *Drug Development & Delivery*. 2012: 30-39.
36. Miller, D. J., Kasemset, S., Wang, L., Paul, D. R. And Freeman, B. D. Constant flux crossflow filtration evaluation of surface-modified fouling-resistant membranes. *Journal of Membrane Science*. 2014; 452:171-183.
37. Moloughney, J. G., And Weisleder, N. Poloxamer 188 (p188) as a membrane resealing reagent in biomedical applications. *Recent Pat Biotechnol*. 2016; 6(3):200-11.
38. Moreno, M., Zurita, E. And Giralt, E. Delivering wasp venom for cancer therapy. *J Control Release*. 182: 13-21.
39. Mori F., Barni S., Pucci N., Rossi ME., de Martino M., Novembre E. Cutaneous adverse reactions to amoxicillin-clavulanic acid suspension in children: the role of sodium benzoate. *Curr Drug Saf*. 2012; 7(2):87–91.
40. Omodamilola, Omoyayi Ibrahim, and Abdullahi Umar Ibrahim. CRISPR technology: advantages, limitations and future direction. *J Biomed Pharm*. 2018; 1.115: 2.

41. Portran, D.). Micropatterning Microtubules. Methods in Cell Biology. Matthieu P, Manuel T (Editors). Academic Press, USA. 2014: 39-51.
42. Qin X. Etiology of infl ammatory bowel disease: a unified hypothesis. *World J Gastroenterol*. 2012; 18(15):1708–1722.
43. Santos, J. L., Cordeiro, P. and Temtem, M. Scale-up of spray dried amorphous solid dispersions. *Eur. Indust. Pharm*. 2013; 19:4-8.
44. Setthacheewakul, S., Kedjinda, W., Maneenuan, D., Wiwattana-patapee, R. Controlled release of oral tetrahydrocurcumin from a novel self-emulsifying floating drug delivery system (SEFDDS). *AAPS. Pharm. Sci. Tech*. 2011; 12(1):152-164.
45. Shah, N., Iyer, N. R. M., Mair, H, G., Choi, D. S., Tian, H., Diodone, R., Fahnrich, K., Pabst-Ravot, A., Tang, K., Scheubel, E., Grippo, J. F., Moreira, S. A., Go, Z., Mousakountakis, J., Louie, T., Ibrahim, P. N., Sandhu, H., Rubia, L., Chokshi, H., Singhal, D. and Malick W. Improved human bioavailability of vemurafenib, a practically insoluble drug, using an amorphous polymerstabilized solid dispersion prepared by a solvent controlled co-precipitation. *J. Pharm. Sci*. 2013; 102:967-981.
46. Teodorescu, M., Bercea, M. And Morariu, S. Biomaterials of PVA and PVP in medical and pharmaceutical applications: perspectives and challenges, Biotechnol. *Adv*. 2019; 37:109–131. https://doi.org/10.1016/j.biotechadv.2018.11.008.
47. Thokala, P., Devlin, N., Marsh, K., Baltussen, R., Boysen, M. And Kalo, Z. Multiple criteria decision analysis for health care decision making—an introduction:report 1 of the ISPOR MCDA emerging good practices task force. *Value Health*. 2016; 19:1–13. doi: 10.1016/j.jval.2015.12.003.
48. Tong X. and Yang F. Engineering interpenetrating network hydrogels as biomimetic cell niche with independently tunable biochemical and mechanical properties. *Biomaterials*. 2014; 35:1807-1815.
49. Uzun Ozsahin, D., Onakpojeruo, E. P., Uzun, B., Mustapha, M. T., & Ozsahin, I. Mathematical Assessment of Machine Learning Models Used for Brain Tumor Diagnosis. *Diagnostics*. 2023; 13(4): 618.
50. Wieduwild, R., Lin, W., Boden, A., Kretschmer, K., Zhang, Y. A repertoire of peptide tags for controlled drug release from injectable noncovalent hydrogel. *Biomacromolecules*. 2014; 15:2058-2066.
51. Xu, J., Feng, E, And Song, J. Bioorthogonally Cross-Linked Hydrogel Network with Precisely Controlled Disintegration Time over a Broad Range. *J Am Chem Soc.* 2014; 136: 4105-4108.
52. Yu X, Buevich AV, Li M, Wang X, Rustum AM A compatibility study of a secondary amine active pharmaceutical ingredient with starch: identification of a novel degradant formed between desloratadine and a starch impurity using LC-MS(n) and NMR spectroscopy. *J Pharm Sci*. 2012; 102(2):717–731.
53. Zardavas, D., Baselga, J. & Piccart, M. Emerging targeted agents in metastaticbreast cancer. 2013. *Nat. Rev. Clin. Oncol*. 10:191–210

تحسين وسيلة توصيل الدواء باستخدام اختيار المواد المساعدة المبني على قرارات متعددة المعايير (MCDM)

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ملخص

تُستخدم المواد المساعدة في أنظمة توصيل الدواء كوسيلة لتوصيل الأدوية بفعالية إلى موقعها المستهدف. تُعد طرق اتخاذ القرار متعددة المعايير (MCDM) أدوات لاتخاذ القرارات التي يمكن أن تضع في الاعتبار العوامل متعددة الأبعاد بالإضافة إلى التكنولوجيا المقارنة المستخدمة في الطب مع مزيج من المعايير الفردية في التقييم الكلي للبدائل المختارة. تهدف هذه الدراسة إلى تحسين الذوبانية والتوافر الحيوي للأدوية من خلال تطبيق اختيار المواد المساعدة المستند إلى MCDM. من خلال دمج طريقة ترتيب التفضيلات لتقييمات التحسين (PROMETHEE الضبابي)، يمكن تقييم المواد المساعدة المختلفة وترتيبها بناءً على مدى ملاءمتها للتطبيق المحدد، مع الأخذ في الاعتبار المعايير المتعلقة بذوبانية الدواء وتوافره الحيوي. تسلط النتائج الضوء على إمكانات السيكلودكسترين (صافي التدفق: 0.0023) وبوليمرات اليودراجيت (صافي التدفق: 0.0016) كخيارات مفضلة لحاملات الدواء، بينما يتم تحديد بولوكسامر 188 (P188) (صافي التدفق: −0.0030) كأقل خيار مفضل. تُظهر هذه الدراسة فعالية طريقة PROMETHEE الضبابي في تحسين أداء الأدوية ذات الذوبانية والتوافر الحيوي المنخفض، مما يساهم في النهاية في تطوير أنظمة توصيل الدواء الجديدة. تتمتع النتائج بأهمية كبيرة للنتائج العلاجية في علاج الأمراض.

الكلمات الدالة: اتخاذ القرار متعدد المعايير (MCDM)، مادة مساعدة حاملة للدواء، الذوبانية، التوافر الحيوي.

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Evaluation of the Impact of Orange Juice on Apixaban Pharmacokinetics in Healthy Rats

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ABSTRACT

Juice derived from the "sweet orange" cultivar is widely consumed and is considered one of the most popular juices globally. It contains many bioactive compounds that can interact with pharmaceutical agents. This study aimed to assess the impact of oral co-ingestion of orange juice (OJ) and Apixaban (AP) on the fundamental pharmacokinetic characteristics of AP, Cmax, and AUC0-t. Two groups of Wistar rats were used in this study: one was given the drug alone, and the other was given the drug with OJ. Each animal was given 10 ml of freshly squeezed orange juice two hours before the administration of AP at a dose of 5 mg/kg and 10 ml concurrently with it. The plasma samples were withdrawn up to 72 hours later and analyzed using the LC/MS technique, and pharmacokinetic parameters were analyzed using Winnonlin version 8.3. The findings indicated a statistically significant increase in Cmax of AP from 28.12±3.78 ng/mL to 56.97±9.8 ng/mL, as well as an increase in AUC0-12 levels from 285.04±24.5 ng. hr/mL to 827.17±46.58 ng.hr/mL when ingested with OJ, without a significant change in Tmax and half-life (t1/2). The results determined that consuming sweet OJ exhibits a noteworthy interaction with orally administered AP.

Keywords: AP (AP); HPLC; method validation; orange juice (OJ); Citrus sinensis pharmacokinetic interactions.

INTRODUCTION

Over the last two decades, one of the most significant challenges confronting pharmaceutical and clinical studies has been food-drug interactions. This has become a matter of concern for many researchers, especially when it relates to life-saving or narrow therapeutic window medications [1,2].

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Food-drug interactions can be defined as changes in the pharmacokinetics and/or pharmacodynamics of a medication or any dietary component, or they may compromise nutritional status associated with, or as a result of, combining food and medication [3,4,5]. Older patients and those with chronic diseases are particularly at risk as this population group consume more than one-third of prescribed medications [6]. Consequently, failure to identify drug-food interactions may lead to complex or harmful consequences [7].

Therefore, food-drug interactions can lead to a

decrease in the absorption of some oral medications and may result in a suboptimal concentration of the drug at the site of action. Ultimately, it can lead to an unfortunate failure of the patient's treatment process [8].

The inhibition or induction of chemical mediators, specifically enzymes present in the gut through food nutrients, can lead to significant changes in the pharmacokinetics and bioavailability of many medications [9]. The most common example is grapefruit juice, which specifically inhibits CYP3A4 in the intestine. This can result in an over fivefold increase in the total exposure to some medications, such as talinolol, when taken with grapefruit juice [10]. Consequently, there is an increased risk of unwanted side effects. Certain medications can impair gastrointestinal functionality and cause electrolyte and fluid losses. To mitigate these unfavorable drug-nutrient interactions, it is important to restrict the use of prescribed medications to those strictly necessary and to regularly reevaluate prescribed therapies [11].

Uncontrolled consumption of a large quantity of sweet orange juice (OJ) may decrease the absorption of celiprolol (Celicard), thereby reducing its effectiveness. To avoid this interaction, a minimum of four hours should separate the consumption of the medication and the juice [12].

Vanapalli et al. reported a reduction in the oral bioavailability of ivermectin when consumed with orange juice (OJ). In their study involving 16 healthy volunteers, 150µg/kg of ivermectin was administered with either water or OJ (750 mL over 4 hours), followed by plasma analyses up to 72 hours later. Their findings indicated a significant reduction in both Cmax and AUC, with Tmax remaining relatively consistent – suggesting a decrease in the extent of drug absorption [13].

Another viewpoint concerns medications transported via cellular pumps. OJ could alter the mechanism or activity rate of these pumps, which in turn could change the amount of medication absorbed by the body. This could potentially make these drugs either less or more active. Lilja et al. observed several interactions of this kind with anti-allergic and other medications such as fexofenadine, levofloxacin, celiprolol, and atenolol [14].

Neuhoff et al. reported a significant 23% reduction in ciprofloxacin's Cmax when given with OJ, with an even steeper 41% reduction when consumed with calcium-fortified OJ. The AUC of ciprofloxacin over 24 hours diminished by 38% and 22% with each form of the OJ respectively [15]. Kamath et al. further reported that sweet orange might decrease the amount of fexofenadine absorbed by the body [16].

Such research has numerous limitations due to factors like the absence or deficiency of complete evidence or information, inaccuracies in medical reports, significant lack of clinical documentation, and awareness about the effect food may have on drug pharmacodynamics or pharmacokinetics. Therefore, studying food-drug interactions from every perspective is crucial – even when there are no reports on the topic, or concerns directly linked to the medicine being studied – especially when such a drug falls into the narrow therapeutic window or critical medications, as previously mentioned [17].

MATERIAL AND METHODS

The following materials were used: Apixaban (with 99.7% purity, from Sigma), AP13C (kindly provided by the Triumpharm Research Center of Clinical Studies, Amman, Jordan), formic acid and acetonitrile (HPLC grade, from Merck, Germany), and sweet oranges from local farms.

2.1 Method Development

Apixaban (AP) in rat plasma was detected and quantified using HPLC/MS. The optimal mobile phase for AP separation was found to consist of acetonitrile, deionized water, and formic acid in a 70:30:0.1 ratio (v/v). An internal standard, AP 13C, was employed. The analysis was conducted using a suite of Shimadzu instruments, including an SCL-10A VP system controller, LC-10AT VP pump, SIL-10AD VP auto-injector with a sample cooler, DGU-14A VP degasser, and SPD-10A VP

ultraviolet detector (all supplied by Kyoto, Japan). Data were collected and processed using Shimadzu VP software (version 5.03). The analytical column was an ACE C18 column, measuring 4.6x100mm with a 5.0µm particle size.

2.2 Extraction Method

Unknown samples and/or spiked plasma were thawed at room temperature before pipetting and were mixed until homogeneous. A total of 30 µL of the internal standard (0.5 g of AP 13C in D3/mL) and 600 µL of the precipitation agent (methanol or MeOH) were added to the tube containing 100 µL of the blank or spiked plasma. The mixture was vortexed for 5 seconds, followed by an additional minute of vortexing. After centrifuging for 7 minutes at 14,000 rpm, approximately 300 µL of each sample was injected into a glass vial with a flat-bottom insert.

2.3 Method Validation

Validation was conducted following the International Council for Harmonisation (ICH) guidelines and acceptance criteria. Aspects such as matrix effect, recovery, linearity, precision, accuracy, and detection limit were optimized in the method.

2.3.1 Matrix Effect

Matrix effects often occur due to alterations in the ionization efficiency of target analytes, typically in the presence of co-eluting substances within the same matrix [18]. This can either increase or decrease the response due to enhancement or suppression of the ionization state. For this reason, the labeled internal standard (IS) was employed in this method and calculated by

$$\textbf{Equation 1: MF of the analyte} = \frac{\textbf{peak area in the presence of matrix}}{\textbf{peak area in the absence of matrix}}$$

$$\textbf{Equation 2: IS} - \textbf{normalized Matrix effect (MF)} = \frac{\textbf{MF of analyte}}{\textbf{MF of internal standard}}$$

2.3.2 Precision, Accuracy, and LLOQ (lower limit of quantification)

The method's accuracy was determined by comparing the actual quantities recovered from the control samples with the expected values present in the samples (theoretical values) [19]. As per the ICH guidelines, the permissible relative standard deviation (RSD) limit should be less than 15%. The QC_{low} (3 ng/mL), QC_{MID1} (24 ng/mL), QC_{MID2} (80 ng/mL), and QC_{High} (160 ng/mL) samples were extracted.

2.3.3 Linearity and Calibration Curve

The Apixaban (AP) Calibration Curves and Linearity Test Concentrations were set at 1, 5, 10, 20, 50, 100, and 200 ng/mL. The Lower Limit of Quantification (LLOQ) was also calculated.

2.3.4 Recovery

Recovery is the percentage of the quantity of an analyte existing in or added to the analytical component of the test material that is extracted and prepared for measurement (ICH guidelines), as illustrated in Equation 3.

$$\textbf{Equation 3: Recovery } \% = \frac{\textbf{Area of extracted plasma sample}}{\textbf{Area of blanks spiked with the analyte post extraction}} \times \textbf{100}\%$$

2.3.5. Method Selectivity

The method's selectivity was assessed by evaluating the blank sample (plasma) and the experiment's zero samples (immediately prior to medication administration).

2.4 Pharmacokinetic Study

2.4.1.Animals and Dosing

The study protocol was approved by the ethics review board. The research utilized Wistar rats weighing approximately 200±15 g, all of which were male and 8 weeks old. The animals were prepared for the experiment by fasting overnight (roughly 12 hours), with water access only. All rats were identified by tail tags before being divided into two groups, each containing six rats. Group 1 received an oral dose of AP (prepared in a distilled water suspension) at 5 mg/kg, whereas Group 2 was given 10 mL of orange juice (Citrus sinensis) both two hours prior to the experiment and concurrently with an oral dose of AP (prepared in a distilled water suspension) at 5 mg/kg.

2.4.2 Preparation of Doses and Juice

AP doses were prepared by suspending 10 mg of AP in 10 mL of distilled water and stirring before administration. The linear pharmacokinetics of AP was confirmed by Frost et al. [20]. The analysis method was designed in a range of 1-200 ng/mL, which made detecting smaller concentrations after administration and during elimination challenging. Each animal received the designated dosage by oral gavage. Freshly squeezed orange juice was made using a press squeezer and measured with a volumetric cylinder before being administered to the animal by oral gavage. The fruits used were purchased from Al-Gour, near the Jordan River, and were freshly picked in February 2021.

2.4.3 Samples of Plasma

Samples were collected from the rat tail. Before each collection, the rat tail was warmed for half a minute using a hot pad. A total of 250 µL was drawn at the following time intervals: zero, 20, 40, 1.5, 3, 5, 8, 12, 24, 36, and 72 hours. Blood samples were drawn into heparinized tubes, centrifuged for 15 minutes at 3500 RPM to separate the plasma, and then frozen at -25 °C for subsequent analysis.

2.4.4 Non-compartmental analysis (NCA) of plasma Concentration-time Data

The basic bioavailability parameters used in this study were the maximum drug concentration in plasma (Cmax), observed time to reach maximal concentration (Tmax), drug elimination rate constant derived from the slope of the later points in the elimination phase (Kel), and AUC0-t. These parameters were calculated using Winnonlin software (version 8.3), and statistical comparisons were performed using ANOVA with a 10% CI as the significance limit.

3. RESULTS

3.1 Method Development and Validation

AP was measured in rat plasma samples using the described technique. A sample of AP chromatograms is shown in Figure 1, with a retention time of 1.08 minutes (A), and (B) represents the blank plasma.

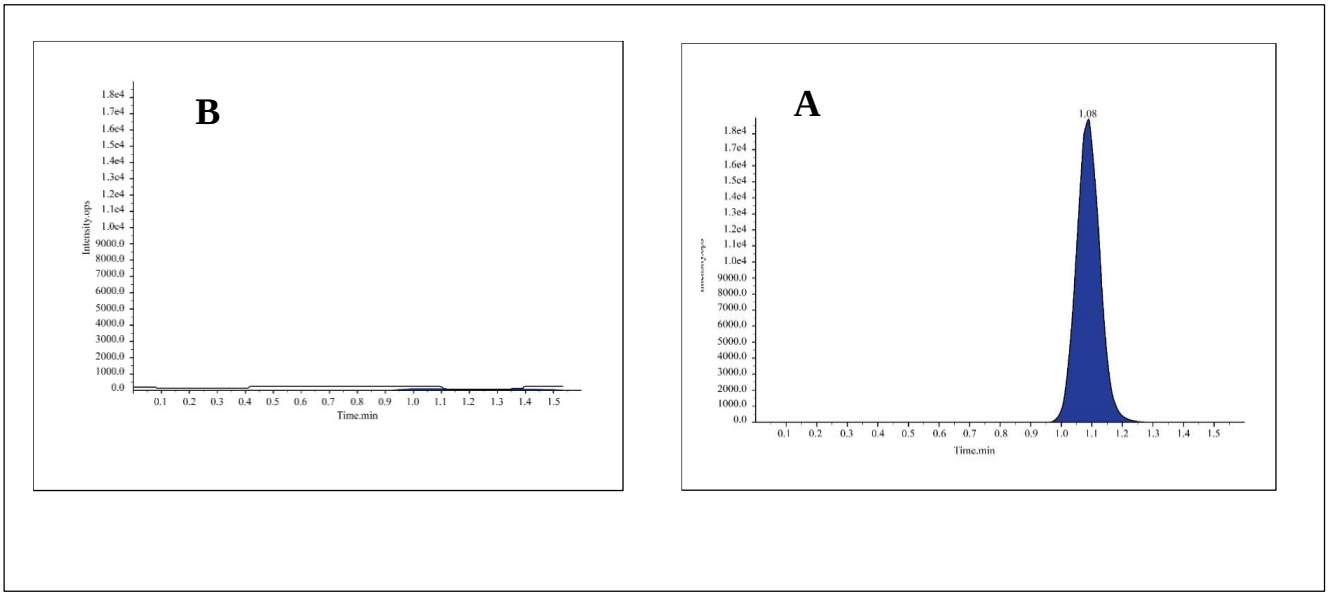

Figure 1: Chromatogram of AP (A blank plasma B AP showing retention time at 1.08 minutes)

3.2 Method Validation

3.2.1 Matrix Effect: The matrix effect results for Apixaban (AP) indicated that the calculated Relative Standard Deviation (RSD) was less than 15%, adhering to the limit set by the ICH guidelines.

3.2.2 Precision, Accuracy, and (lower limit of quantification) LLOQ

An acceptable accuracy range of 85–115% was achieved for all concentrations [21]. The "within-run" accuracy results display that AP in plasma can be quantified with sufficient accuracy and precision across the entire concentration spectrum. As the results from the LLOQ demonstrate, quantities larger than the LLOQ can be precisely and accurately quantified using the AP analytical method. The results are provided as supplementary data.

3.2.3 Linearity and Calibration Curve

Using the mean of three linearity test runs, AP concentrations ranging from 5.00 ng/mL to 200 ng/mL yielded a linear regression with a correlation coefficient of R = 0.9992, demonstrating the linearity of the concentration used with the area under the curve (AUC) measured for the drug and internal standard (IS). This is depicted in Figure 2. The RSD of back calculation was as follows: 12.64 (1 ng/mL), 1.41 (5 ng/mL), 5.25 (10 ng/mL), 7.84 (20 ng/mL), 2.27 (50 ng/mL), 6.98 (100 ng/mL), and 2.77 (200 ng/mL). The LLOQ was determined to be 2 ng/mL.

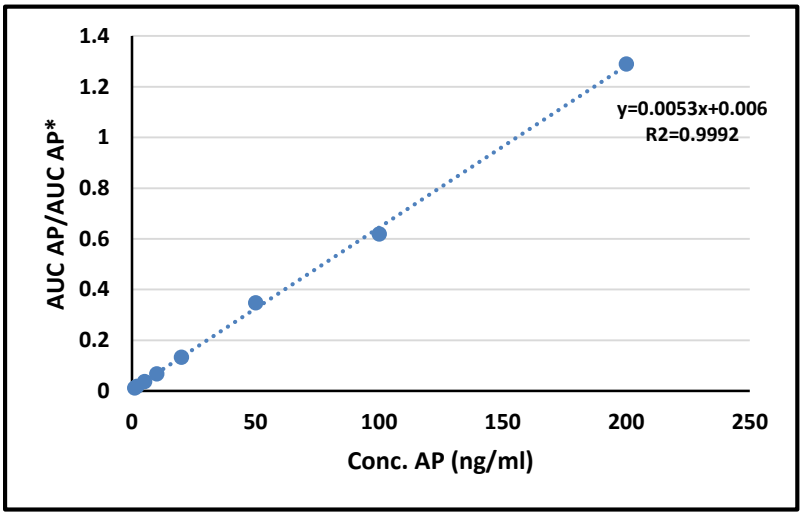

Figure 2: Calibration curve of AP

3.2.4 Recovery

As per the International Council for Harmonisation (ICH) guidelines for measuring Apixaban (AP) concentrations in rat plasma, the method was sufficiently reliable for pharmacokinetic studies. The average recovery was 85.13% with a relative standard deviation (RSD) of 5.72%, satisfying the guideline criteria of 85-115%. These results are presented in Table 1. The methodology's selectivity was confirmed, as no AP peak was detected in the blank plasma.

Table 1: Results of the recovery test.

Replicate	IS (Peak Area) in QC $_{Low}$ (Peak Area)		IS (Peak Area) in QC_{Med-1} (Peak Area)		IS (Peak Area) in QC_{Med-2} (Peak Area)		IS (Peak Area) in QC $_{High}$ (Peak Area)	
	Extracted	Post Spiked	Extracted	Post Spiked	Extracted	Post Spiked	Extracted	Post Spiked
1	65246	67151	64293	69782	62828	79096	60947	77844
2	61481	69647	59061	72886	64377	74397	63556	77851
3	60775	67771	59816	70956	59171	71847	61234	75292
Mean	**62501**	**68190**	**61057**	**71208**	**62125**	**75113**	**61912**	**76996**
Recovery %	**91.66**		**85.74**		**82.71**		**80.41**	
Mean	**85.13**							
SD	**4.870**							
RSD	**5.72**							

3.3 Pharmacokinetic Study

3.3.1 Construction of Plasma Level Time Profiles

The Cmax and AUC were higher in group 2 than in group 1, as depicted in Figure 3. Plasma analysis at 24, 36, and 72 hours yielded no results as they fell below the Lower Limit of Quantification (LLOQ). Therefore, the results are presented up until the 12-hour mark.

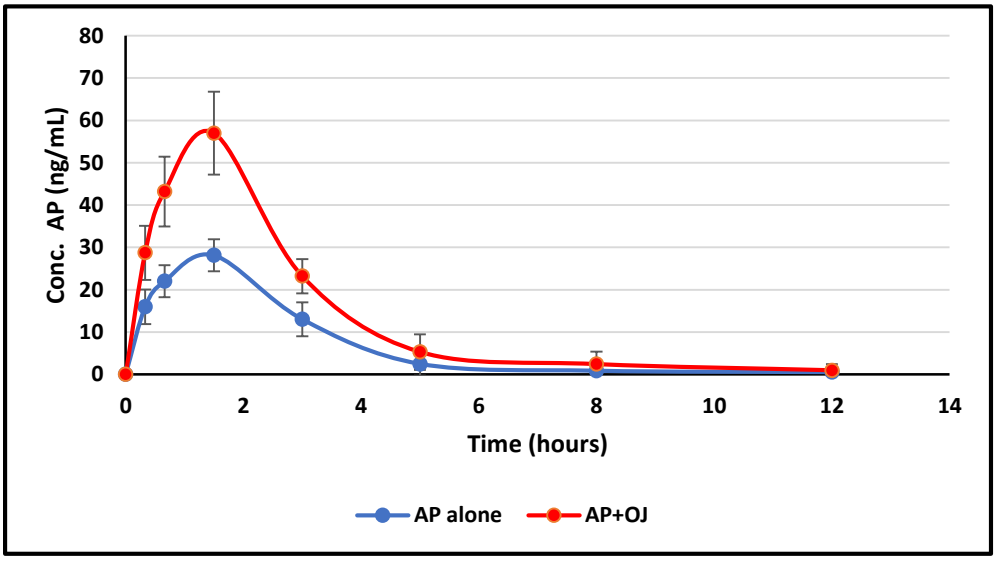

Figure 3: Plasma level-time profile of AP in rats' plasma (G1"AP only" and G2 "AP with OJ").

3.3.2 Calculation of Pharmacokinetic Parameters

Table 2 shows the pharmacokinetic parameters of AP with and without OJ. Statistical analysis showed that C_{max} increased significantly as well as AUC_{0-12}, while elimination rate constant (Kel) and half-life ($t_{1/2}$) showed non-significant difference in their values by ANOVA.

Table 2. AP basic pharmacokinetic characteristics in the examined groups

Group no.	C_{max} ±SD (ng/mL)	T_{max} (hr) (median)	AUC_{0-12} ±SD (ng. hr.mL^{-1})	Kel ±SD (h^{-1})	Half-life $T_{1/2}$ (h)
G1, AP alone	28.12±3.78	1.5	285.04 ± 24.5	0.0698 ± 0.098	10.07±0.6
G2, AP+ OJ	56.97 ±9.8 *	1.5	827.17 ± 46.58*	0.0687 ± 0.085	10.08±0.92

*** significant (0.1 CI)**

4. DISCUSSION

The Cmax of Apixaban (AP) alone was found to be 28.12±3.78 ng/mL and increased to 56.97±9.8 ng/mL when consumed with orange juice (OJ). ANOVA results revealed that the increase in Cmax with OJ was statistically significant (CI = 0.1). Although Cmax is an extent and rate parameter, without knowing Tmax and AUC, it's impossible to determine whether the interaction is due to the quantity or rate of drug absorption. The variance could have been affected by a change in pace, magnitude, or both. The Tmax (median) was 1.5 hours (90 min) for both groups, indicating no difference between them. This suggests that the absorption rate remained constant, with the same amount of AP being absorbed no matter how it was taken. When administered alone, AP has an AUC_{0-t} of 285.04±24.5 ng.hr/mL, which significantly increased to 827.17±46.58 ng.hr/mL when consumed with citrus sinensis OJ, as depicted in Figure 3. This implies that more AP was absorbed when taken with OJ, resulting in a larger Cmax. The elimination rate constant (Kel) for AP was calculated for both groups, and the results showed no significant difference in Kel between the two groups. This indicates that OJ did not affect the elimination pattern of AP.

Interactions between food, juices, and drugs at the site of absorption frequently occur at three levels: the first and most common being intestinal cytochrome P3A4, the second being intestinal uptake of organic anion-transporting polypeptides (OATPs), specifically OATP2B1, and thirdly, the efflux transporter, P-glycoprotein (P-GP) [22]. This study revealed that when orange juice (OJ) was co-administered with Apixaban (AP), there were significant increases in the Cmax and AUC of the drug. This could potentially be due to OJ inhibiting the P-GP efflux mechanism [23], leading to greater drug absorption and thus a higher Cmax and AUC. The drug absorption rate remained consistent between both groups, indicating that the interaction primarily influenced the extent of absorption, as manifested by the higher AUC0-t.

Breast cancer resistance protein (BCRP, gene ABCG2), an efflux transporter present in numerous healthy tissues, including the gastrointestinal tract, colon, liver, and kidneys, can reduce systemic exposure to several drugs — including AP — since BCRP acts as rate-limiting barrier to the absorption of its substrates from the intestine and enhances the excretion of its substrates into the bile and urine [24, 25].

This study lends further support to the growing evidence that efflux contributes to AP's low oral bioavailability. Honda et al. found in their research on Caco-cells that OJ inhibits vinblastine transport from apical to basal membrane through interaction with P-GP and multidrug resistance protein (MRP2), both found in apical membranes [26]. As AP is predominantly eliminated through bile excretion [27], the unchanged elimination rate throughout the study (identical Tmax in both groups) suggests that OJ primarily affected drug absorption and had no impact on AP's elimination. Therefore, when AP is co-administered with OJ as an oral anticoagulant, clinical monitoring is necessary, chiefly for bleeding symptoms such as hemorrhage and hematoma, in response to the anticipated increase in systemic AP concentration. Thus, concurrent administration of OJ from Citrus sinensis had a significant impact, namely an increase, on the extent of AP absorption.

CONCLUSION

A validated, rapid, and sensitive analytical method has

been successfully developed for Apixaban (AP) in healthy rat plasma, ideally suited for pharmacokinetic applications. The results of this study demonstrate that consumption of orange juice (Citrus sinensis) alongside AP results in increased absorption and bioavailability of AP, without any significant alteration in AP's elimination.

Ethical Approval

The study protocol was approved by the Ethics Review Board at Al-Ahliyya Amman University, as per decision no. (AUP: AAU/1/4/2021-2022), Faculty of Pharmacy, Al-Ahliyya Amman University, Jordan.

Acknowledgment

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Declaration of conflict

The authors declare that they have no conflicts of interest.

REFERENCES

1. Matzke G R., Aronoff G R., Atkinson A J., Bennett W M., Decker B S., Eckardt K. U., Golper T., Grabe D W., Kasiske B., Keller F., Kielstein J T., Mehta R., Mueller B A., Pasko D A., Schaefer F., Sica D A., Inker L A., Umans J G., and Murray P. Drug dosing consideration in patients with acute and chronic kidney diseasea clinical update from Kidney Disease. Improving Global Outcomes (KDIGO). *Kidney. Int.* 2011; 80(11):1122–1137.
2. Palleria C., Di Paolo A., Giofrè C., Caglioti C., Leuzzi G., Siniscalchi A., De Sarro G., and Gallelli, L. Pharmacokinetic drug-drug interaction and their implication in clinical management. *J. Res. Med. Sci*. 2013; 18(7):600–609.
3. Almomani E Y., Jarrar W., Alhadid A., Hamadneh L., Qablan A., and Almomani, H. Y. Shedding light on pharmacists' knowledge of kidney stones' etiology and treatment. *J. Pharm. Pract*. 2022; 20(3).
4. Koziolek M., Alcaro S., Augustijns P., Basit A W., Grimm M., Hens B., Hoad C. L., Jedamzik P., Madla C M., Maliepaard M., Marciani L., Maruca A., Parrott N., Pávek P., Porter C J H., Reppas C., van Riet-Nales D., Rubbens J., Statelova M., … Corsetti M. The mechanisms of pharmacokinetic food-drug interactions – A perspective from the UNGAP group. *Eur. J. Pharm. Sci*. 2019; 134:31–59.
5. Abu Dayyih W., Mallah EM., Al-Ani I., Arafat T. Liquorice Beverage Effect on The Pharmacokinetic Parameters Of Atorvastatin, Simvastatin, And Lovastatin By Liquid Chromatography-Mass Spectroscopy/Mass Spectroscopy. *Asian J. Pharm. Clin. Res*. 2016; 9(2):143-179.
6. Miller N H. Compliance with treatment regimens in chronic asymptomatic diseases. *Am. J. Med*. 1997; 102(2A):43–49.
7. Paudel S., Palaian S., Shankar P R., and Subedi N. Risk Perception and Hesitancy Toward COVID-19 Vaccination Among Healthcare Workers and Staff at a Medical College in Nepal. Risk. Manag. *Health Policy*. 2021; 14: 2253–2261.
8. Cheng L., and Wong H. Food Effects on Oral Drug Absorption: Application of Physiologically-Based Pharmacokinetic Modeling as a Predictive Tool. *Int. J. Pharm*. 2020; 12(7):1–18.
9. Bushra R., Aslam N., and Khan A Y. AP: A Clinical Pharmacokinetic and Pharmacodynamic Review, 58. Clinical. Pharmacokinetics. 2019. Food-drug interactions. Oman. *Med. J.* 2011; 26(2):77–83.
10. Genser D., Food and drug interaction: consequences for the nutrition/health status. *Ann. Nutr. Metab*. 2008; 52 (1): 29–32.

11. Rodriguez-Fragoso, Lourdes, and Jorge Reyes-Esparz. 'Fruit/Vegetable-Drug Interactions: Effects on Drug Metabolizing Enzymes and Drug Transporters'. Drug. Discov. *InTech*, 2013.
12. Alam M.A., Subhan N., Rahman M.M., Uddin S.J., Reza H M., Sarker, S. D. Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. *Adv. Nutr*. 2014; 5(4):404–417.
13. Vanapalli, S. R., Chen, Y., Ellingrod, V. L., Kitzman, D., Lee, Y., Hohl, R. J., & Fleckenstein, L. Orange juice decreases the oral bioavailability of ivermectin in healthy volunteers. *Clin. Pharmacol. Ther*. 2003; 73(2): 94–94.
14. Lilja J.J., Raaska K., Neuvonen, P.J. Effects of OJ on the pharmacokinetics of atenolol. *European Journal of Clin. Pharmacol*. 2005; 61:337–340.
15. Neuhofel A.L., Wilton J.H., Victory J.M., Hejmanowski L.G., Amsden G.W. Lack of bioequivalence of ciprofloxacin when administered with calcium-fortified orange juice: a new twist on an old interaction. *J. Clin. Pharmacol*. 2002; 42(4):461–466.
16. Kamath A.V., Yao M., Zhang Y., Chong S. Effect of fruit juices on the oral bioavailability of fexofenadine in rats. *Jordan j. pharm. Sci.* 2005; 94(2):233–239.
17. Williamson E M. Drug interactions between herbal and prescription medicines. *Drug. Saf.* 2003; 26(15):1075–1092.
18. AbuDayyih WA, Manaysa MH, Hailat M., Zakareia Z., El Hajji F. Influence of Castor Oil on Glycated Hemoglobin (HbA1c) on Induced Type 2 Diabetes Mellitus in Rats. *Jordan j. pharm. Sci*. 2021; 14(3):341-349.
19. Sa`adeh A., Aburjai T., Shraim N., Al-Ghazawi M. The Effect of Brassica Oleracea (Cabbage) on The Pharmacokinetics of Ciprofloxacin in an Animal Model. *Jordan j. pharm. sci*. 2019; 12(2): 59–67.
20. Frost C, Shenker A, Jhee S, et al. Evaluation of the single-dose pharmacokinetics and pharmacodynamics of apixaban in healthy Japanese and Caucasian subjects. *Clin Pharmacol*. 2018; 10:153-163.
21. Habash I W., Al-Shdefat R I., Hailat M M., Dayyih W A., and Abu Dayyih W A. stability indicating RP-HPLC method development for simultaneous estimation of alogliptin, pioglitazone, and metformin in pharmaceutical formulations. *Acta. Pol. Pharm*. 2020; 77(4):549–562.
22. Murakami T, Takano M. Intestinal efflux transporters and drug absorption. *Expert Opin Drug Metab Toxicol*. 2008; 4(7):923-939.
23. Honda Y, Ushigome F, Koyabu N, et al. Effects of grapefruit juice and OJ components on P-glycoprotein- and MRP2-mediated drug efflux. *Br J Pharmacol*. 2004; 143(7):856-864.
24. Wang L., He K., Maxwell B., Grossman S J., Tremaine L M., Humphreys W G., and Zhang D. Tissue distribution and elimination of [14C] AP in rats. *Drug. Metab. Dispos*. 2011; 39(2): 256–264.
25. Hellwig T., and Gulseth M. Pharmacokinetic and pharmacodynamic drug interactions with new oral anticoagulants: what do they mean for patients with atrial fibrillation? *Ann Pharmacother*. 2013; 47(11):1478–1487.
26. Honda Y., Ushigome F., Koyabu N., Morimoto S., Shoyama Y., Uchiumi T., Kuwano M., Ohtani H., and Sawada Y. Effects of grapefruit juice and OJ components on P-glycoprotein- and MRP2-mediated drug efflux. *Br. J. Pharmacol*. 2004; 143(7): 856.
27. Byon W, Garonzik S, Boyd RA, Frost CE. AP: A Clinical Pharmacokinetic and Pharmacodynamic Review. *Clin. Pharmacokinet*. 2019; 58(10):1265-1279.

تقييم تأثير عصير البرتقال على الحرائك الدوائية للأبيكسابان في الفئران السليمة

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ملخص

يستهلك العصير المشتق من صنف "البرتقال الحلو" على نطاق واسع ويعتبر من أكثر العصائر شعبية على مستوى العالم. أنه يحتوي على العديد من المركبات النشطة بيولوجيا التي يمكن أن تتفاعل مع العوامل الصيدلانية. تهدف هذه الدراسة إلى تقييم تأثير تناول عصير البرتقال والأبيكسابان عن طريق الفم على الخصائص الدوائية الأساسية للأبيكسابان. تم استخدام مجموعتين من فئران الوستار في هذه الدراسة، أعطيت إحداهما الدواء بمفرده والأخرى مع عصير البرتقال. تم تحليل عينات البلازما باستخدام تقنية LC/MS وتم تحليل المعلمات الدوائية باستخدام WinNoline الإصدار 8.3. أشارت النتائج إلى ارتفاع ذو دلالة إحصائية في Cmax لـ Apixaban من 28.12 ± 3.78 نانوغرام / مل إلى 56.97 ± 9.8 نانوغرام / مل، بالإضافة إلى زيادة في مستويات AUC0-12 من 285.04 ± 24.5 نانوغرام. ساعة/مل إلى 827.17±46.58 نانوغرام ساعة/مل عند تناوله مع عصير البرتقال. حددت النتائج أن تناول عصير البرتقال الحلو يظهر تفاعلًا ملحوظًا مع أبيكسابان الذي يتم تناوله عن طريق الفم.

الكلمات الدالة: الابكسبان، التحليل اللوني عالي الكفاءة، مقايسة الطريقة، عصير البرتقال، معلّمات الحرائك الدوائية.

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A Review on Recent Advances of Natural Products as Larvicides in Vector Control Management

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ABSTRACT

The mosquito, a biological vector, is responsible for the transmission of serious and dreaded diseases worldwide. These diseases, which are chiefly endemic to tropical countries, cause millions of deaths each year. The significance of plant-based and environmentally friendly insecticides has increased in recent years. Due to their easy biodegradability and target selectivity, they can be used safely in aquatic environments. Despite their effectiveness in controlling target vector species, pesticide applications pose a threat as they can lead to increased chemical insecticide resistance, causing a rebound in vectorial capacity. This review explores the efficacy of phytochemicals in controlling mosquito populations. In mosquito control programs, phytochemicals play a significant role. Plants serve as an immense repository for primary and secondary metabolites. Various types of polar and nonpolar solvents can be used to extract the bioactive plant ingredient(s) from either the whole plant or a specific part of it. This literature review defines natural products and provides an overview of the different types of natural products that can be used to control mosquito larvae. Particularly, it examines the effectiveness of natural products in vector control without causing resistance or harm to non-target organisms. The purpose of this paper is to offer a comprehensive review of the use of natural products as mosquito larvicides and to underscore their potential as an alternative to traditional chemical methods. Ultimately, it encourages further research into the development and use of natural products for successful vector mosquito control.

Keywords: Vector Control, Traditional Method, Natural Products, Phytochemicals.

INTRODUCTION

The mosquito represents a severe threat to public health, transmitting several dangerous diseases like malaria, filaria, dengue, dengue fever, and Japanese encephalitis, primarily in the tropics and subtropics (WHO, 2012). A noticeable increase in these vectors' insecticide resistance has evolved into a global problem. To prevent the transmission of these deadly diseases, mosquito population control is necessary. Chemical insecticides were favored a decade ago, but due to their persistence in the environment and harm to non-target organisms, these have shifted researchers' focus toward the search for new, safer natural products [1]. Natural products, such as plant-based insecticides, have potential for use in mosquito control. These natural products, deemed potential sources of mosquito larvicides due to their low toxicity to non-target organisms and the mosquito's declining resistance to synthetic insecticides, have recently been reviewed [2]. Recent advances in this field include using essential oils derived from plants such as citronella, eucalyptus, and peppermint as larvicides. These oils have demonstrated promising larvicidal activity against different mosquito species. Chitosan, a

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polysaccharide derived from crustacean shells, represents another promising natural product; it has been found to possess strong larvicidal activity against Aedes and Anopheles mosquitoes. Several phytochemicals, such as alkaloids, steroids, terpenoids, essential oils, and phenolics, from various plant parts, have been reported to exhibit insecticidal activities [3,4]. Overall, the use of natural products as mosquito larvicides is a promising approach that could provide an alternative to synthetic insecticides. This review article places emphasis on collating and updating the use of various phytochemicals as mosquito larvicides.

Traditional methods of mosquito control:

Traditional methods of mosquito control have been utilized for many years and involve various techniques to reduce mosquito populations. Here are some examples of traditional mosquito control methods:

- **Source reduction:**

This method involves removing or modifying mosquito breeding sites, such as stagnant water, to prevent the development of mosquito larvae. This can be achieved using techniques such as filling in puddles, draining standing water, and cleaning up trash or debris that can collect water.
(https://www.cdc.gov/zika/vector/source-reduction.html).

- **Larvicidal:**

This method involves the use of insecticides to eliminate mosquito larvae in breeding sites, such as standing water. This can be achieved with a variety of chemicals, including Bacillus thuringiensis israelensis (Bti) and methoprene
(https://www.who.int/water_sanitation_health/resources/vector282to301).

- **Adulticiding:**

This method involves the use of insecticides to kill adult mosquitoes. This can be conducted by spraying insecticides from the ground or the air, or by using fogging or misting devices
(https://www.cdc.gov/zika/vector/mosquito-control.html).

- **Biological control:**

This method involves using natural predators or pathogens to control mosquito populations. For example, introducing fish that eat mosquito larvae into bodies of water can help reduce mosquito numbers (https://www.who.int/neglected_diseases/vector_ecology/vector-control/biological_control/en/).

- **Personal protection:**

This method involves using physical barriers or insect repellents to prevent mosquito bites. This can include wearing long sleeves and pants, using mosquito nets over beds, and applying insect repellents containing DEET or other chemicals (https://www.epa.gov/insect-repellents.).

Limitations of traditional methods:

Traditional methods of mosquito control, such as the use of insecticides and mosquito nets, have some limitations that can hinder their effectiveness in reducing mosquito populations.

Firstly, the repeated use of insecticides can lead to the development of insecticide resistance in mosquitoes, making them less susceptible to the effects of the insecticides [5]. This can reduce the effectiveness of insecticides as a means of controlling mosquito populations.

Secondly, mosquito nets may not be used consistently or correctly, reducing their effectiveness in preventing mosquito bites and the transmission of mosquito-borne diseases [6].

Thirdly, traditional mosquito control methods such as source reduction, larviciding, and adulticiding can be expensive, time-consuming, and require significant resources (World Health Organization, 2019). This can limit their implementation in resource-poor settings.

Finally, traditional mosquito control methods may have unintended environmental consequences, such as the potential to harm non-target species or disrupt ecosystems [7].

Definition of natural products:

Natural products refer to chemical compounds obtained from sources such as plants, microorganisms, and animals, which demonstrate larvicidal activity against mosquitoes. These compounds may act directly on

mosquito larvae, disrupting their physiological processes, or indirectly by interfering with the larvae's growth and development.

One study by [8] defined natural products used as mosquito larvicides as "chemical substances obtained from natural sources that can kill or inhibit the growth and development of mosquito larvae." The authors emphasized the potential of these natural products as a sustainable and environmentally friendly approach to mosquito control.

Types of natural products used as mosquito larvicides:

Several natural products have been examined and employed as mosquito larvicides. We can categorize these natural products based on their chemical structure and origin.

Plant-derived natural products: Several essential oils, alkaloids, flavonoids, and other plant-derived compounds have shown potential as mosquito larvicides. For example, essential oils from plants such as Citronella, Eucalyptus, and Lemongrass have demonstrated larvicidal activity against mosquitoes [9].

Microbial metabolites: Bacteria and fungi produce a variety of secondary metabolites that have demonstrated larvicidal activity against mosquitoes. For instance, the bacterial metabolite Spinosad has been used as a larvicide in mosquito control programs [10].

Marine natural products: Compounds derived from marine organisms such as algae, sponges, and corals have also shown potential as mosquito larvicides. For example, extracts from the marine sponge Acanthella cavernosa have shown to have larvicidal activity against Aedes aegypti [11].

Other natural products: In addition to those already mentioned, other natural products such as neem oil, chitinase, and certain plant extracts have demonstrated larvicidal activity against mosquitoes [12].

Advantages of natural products as mosquito larvicides:

Natural products have attracted substantial attention as alternatives to chemical insecticides in mosquito control programs, due to their environmental friendliness, low toxicity, and cost-effectiveness. Several studies have highlighted the potential of natural products as mosquito larvicides. The advantages include:

- Biodegradability: Unlike synthetic insecticides, which can accumulate in soil and water bodies, leading to pollution and environmental hazards, natural products are biodegradable and do not persist in the environment.
- Selectivity: Natural products are usually more selective, targeting specific insect pests while minimizing the impact on non-target organisms, including humans and other beneficial insects.
- Resistance management: The incidence of resistance development is lower for natural products, compared to synthetic insecticides. Repeated use of synthetic insecticides has led to a rise in resistance amongst mosquitoes, making them less susceptible to chemical control. Natural products, on the other hand, have various modes of action.
- Sustainable: Natural products can be sustainably produced and procured from renewable resources, making them a viable option for long-term use in mosquito control programs.

Some examples of natural products with mosquito larvicidal activity include plant-derived compounds such as alkaloids, flavonoids, and essential oils. Other sources of natural larvicides encompass bacterial and fungal metabolites, as well as extracts from marine organisms.

One study conducted by [13] evaluated the larvicidal activity of essential oils from 20 plant species against Aedes aegypti and found that most of the oils exhibited significant larvicidal activity. Another study by [14] demonstrated the larvicidal potential of a fungal metabolite, cordycepin, against Aedes aegypti and Culex quinquefasciatus.

Below are examples of secondary metabolites of plant origin that have been reported to function as mosquito larvicides.

Alkaloids:

Alkaloids are a diverse group of naturally occurring organic compounds distinguished by their basic (alkaline) properties and nitrogen-containing heterocyclic structures. They are typically derived from about 15% of plant species, though some alkaloids can also be found in fungi and animals. Alkaloids exhibit a wide range of biological activities and are often recognized for their pharmacological effects [15].

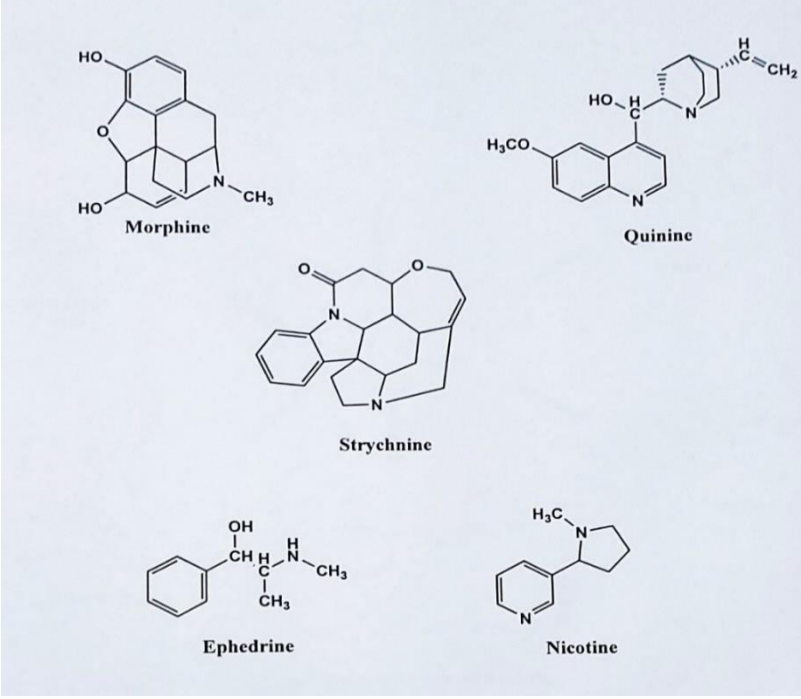


Figure 1: Structure of some alkaloids reported from the plant

Certain plant families among the angiosperms contain a higher concentration of alkaloid-rich species compared to others (Figure 1). Families such as Papaveraceae, Berberidaceae, Fabaceae, Boraginaceae, Apocynaceae, Asteraceae, Liliaceae, Gnetaceae, Ranunculaceae, Rubiaceae, Solanaceae, and Rutaceae are known to include a significant number of taxa that produce alkaloids. These alkaloid-rich plants have been recognized for their potential insecticidal properties, including their effectiveness as mosquito larvicides (Table 1).

Mechanism of action of plant alkaloids:

Plant alkaloids specifically act on microtubule proteins during the metaphase stage of the cell cycle, leading to mitotic arrest. Consequently, the affected cells become incapable of dividing, ultimately leading to their demise. These alkaloids primarily target the M phase of the cell cycle (Figure 2). Their major toxic effects are observed in the hematopoietic, integumentary, neurologic, and reproductive systems.

Quinolizidine alkaloids, on the other hand, impact insects by affecting the potassium channel and glutamate receptor, effectively halting protein synthesis in these organisms. Tetracyclic sparteine, which is derived from Cytisus scoparius, has particularly proven to have insecticidal action [15].

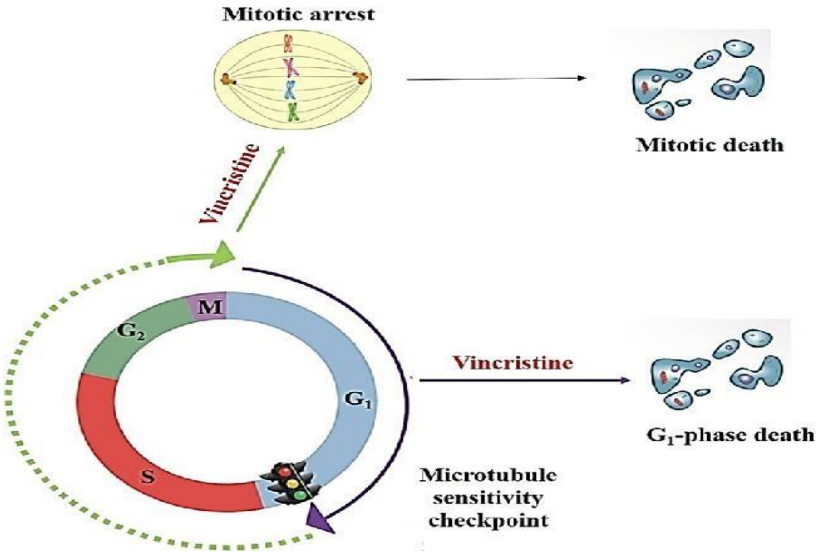


Figure 2: Mode of action of alkaloids [15]

Table 1: Some plant-based alkaloids with mosquito larvicidal properties, along with their respective plant name, family, mosquito species targeted, plant part used, LC_{50} value, and references

S.N.	Plant Name	Family	Mosquito Speciestargeted	Plant Part used	LC_{50} Value	References
1	*Zanthoxylum piperitum*	Rutaceae	*Culex pipiens and Aedes aegypti*	bark	0.006 and 0.009 mg/l respectively	[16]
2	*Mammea americana*	Calophyllaceae	*Aedes aegypti*	seeds	38.58 ppm	[17]
3	*CaricaPapaya*	Caricaceae	*Aedes aegypti*	seeds	At 10% concentraion	[18]
4	*Tinosporarumphii*	Menispermaceae	*Aedes aegypti*	leaf	10 mg/ml	[19]
5	*Murraya koenigii*	Rutaceae	*Aedes aegypti*	root	1.75 µg/ml	[20]
6	*Tridax procumbens*	Asteraceae	*Aedes aegypti*	leaves	219 µg/ml	[21]
7	*Tagetesminuta*	Asteraceae	*Anopheles stephensi*	Aerialpart	2.5 mg/l	[22]
8	*Ruta graveolens*	Rutaceae	*Culiseta longiareolata*	leaves	43.24 ppm	[23]
9	*Lantanacamara*	Verbenaceae,	*Anopheles stephensi*	leaves	36.65 ppm	[24]
10	*Solanum nigrum*	Solanaceae	*Cx. quinquefasciatus*	fruits	48.41µg/ml	[25]

Flavonoids:

Flavonoids are a class of plant compounds that are widely distributed in nature and are known for their diverse biological activities. These compounds have been extensively studied for their antioxidant and anti-inflammatory properties; their potential as mosquito larvicides has also been explored.

One of the mechanisms by which flavonoids exhibit larvicidal activity is through their interference with mosquito larvae's growth and development. Flavonoids can disrupt the molting process and inhibit chitin synthesis—an essential component of the larval

exoskeleton. This disruption hampers the normal development of the larvae, leading to mortality [26].

The most popular solvents for extracting flavonoids include ethanol, combinations of water in various ratios, and natural deep eutectic solvents (NADES). These solvents are chosen for their ability to solubilize moderately polar flavonoids at a relatively low cost, and with minimal negative environmental impact.

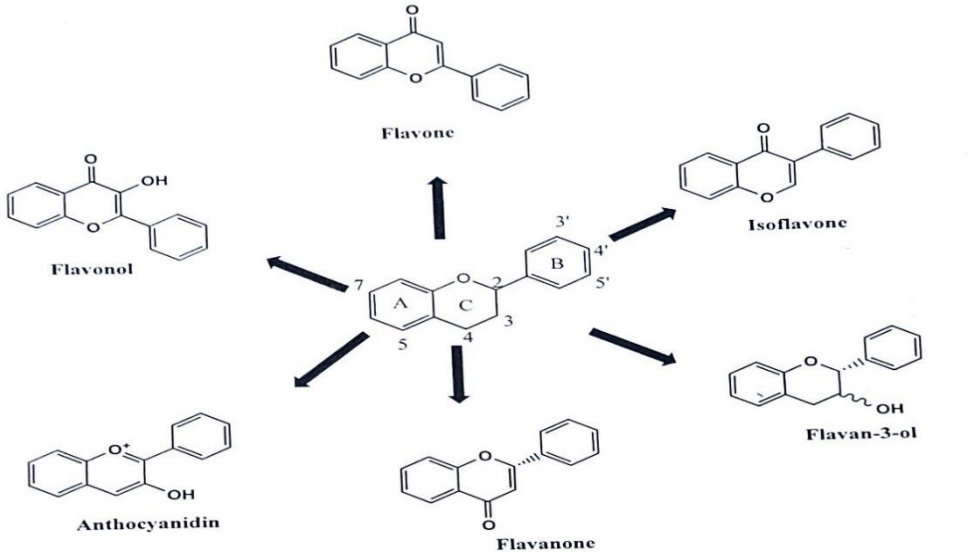


Figure 3: Basic structure of flavonoids

Table 2: Some plant-based Flavonoids with mosquito larvicidal properties, along with their respective plant name, family, mosquito species targeted, plant part used, LC50 value, and references.

S.N.	Plant Name	Family	Mosquito Speciestargeted	Plant Partused	LC50 value	Reference
1	*Vitex negundo*	Lamiaceae	An. Stephensi	root	83.17ppm	[27]
2	*Poncirustrifoliata*	Rutaceae	*Aedes aegypti*	leaves	0.082mg/l	[28]
3	Argemonemexicana	Papaveraceae	*Culex quinquefasciatus*	stem	10.61ppm	[29]
4	*Calotropis gigantea*	Apocynaceae	*Ae. Aegypti*	flower	0.21µg/ml	[30]
5	*Citrus grandis*	Rutaceae	*Ae. Aegypti*	fruit peel	236.70 µg/mL	[31]
6	Artemisia absinthium	Asteraceae	Cs. Longiareolata	leaves	97.74 ppm	[32]
7	*Cassia Occidentalis*	Fabaceae	*Anophelesstephensi*	leaves	64.76	[33]
8	Couroupitaguianensis	Lecythidaceae	*A. aegypti*	leaf	44.55 ppm	[34]
9	*Tagetespatula*	Asteraceae	*Aedes aegypti*	flower	15.74 µg/ml	[35]
10	Anacardium occidentale	Anacardiaceae	Ae. aegypti.	stem	1200 mg/L	[36]

Flavonoids are a group of phytochemicals commonly found in plants. They are known for their diverse biological activities and have been extensively studied for their potential health benefits. While flavonoids have been investigated for their insecticidal properties, their effects on mosquitoes are shown in Table 2.

Flavonoids are a diverse group of compounds found in various plant families, as depicted in Figure 3. Plant families reported to contain flavonoids include Fabaceae, Asteraceae, Rosaceae, Rutaceae, Solanaceae, among others.

Steroids:

Steroid phytochemicals are naturally occurring compounds found in plants and have been investigated for their potential use as mosquito larvicides. Mosquito larvicides are substances designed to control mosquito populations by targeting larvae in their aquatic breeding sites.

Primary plant steroids, known as phytosterols or plant sterols, are depicted in Figure 4. Phytosterols are found in various parts of plants, with the highest concentrations typically found in seeds and fruits [37].

The extraction of phytosterols from plant parts involves several steps, such as harvesting the plant material, crushing or grinding it to increase the surface area, followed by solvent extraction or other separation techniques to isolate phytosterols [38, 39].

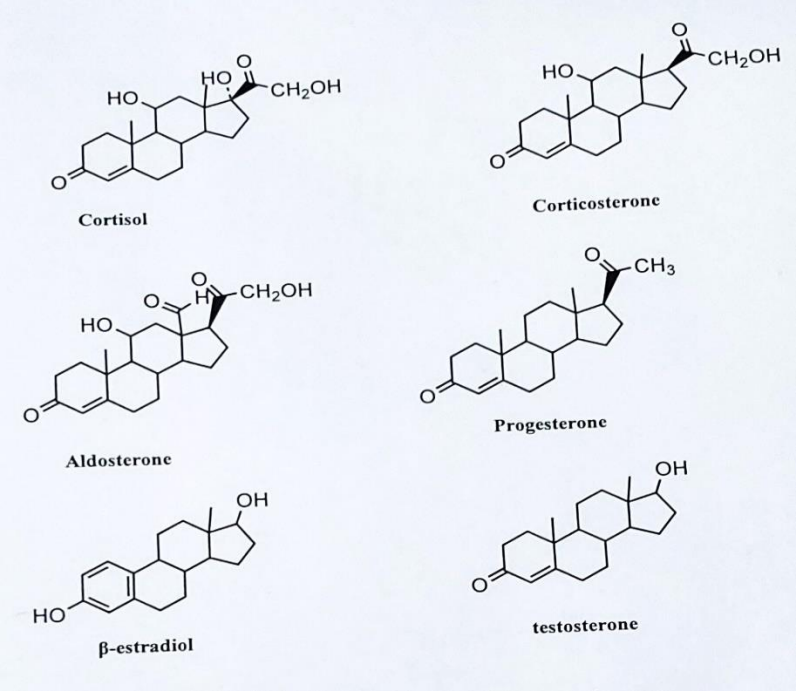


Figure 4: Basic structure of steroids.

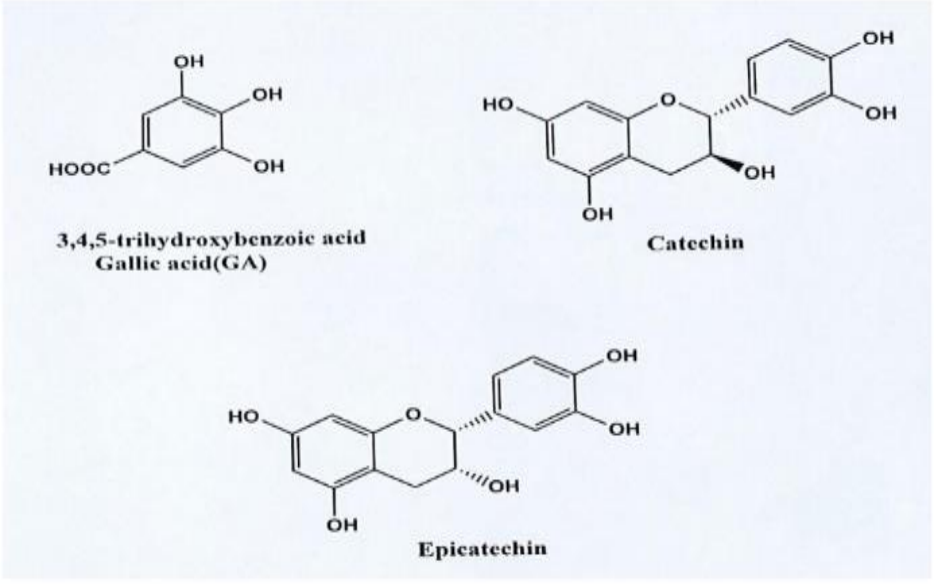
Several plant families, including Solanaceae, Liliaceae, Fabaceae, Asperagaceae, Dioscoreaceae, and Poaceae, are known for producing steroids with mosquito larvicidal activity, as shown in Table 3.

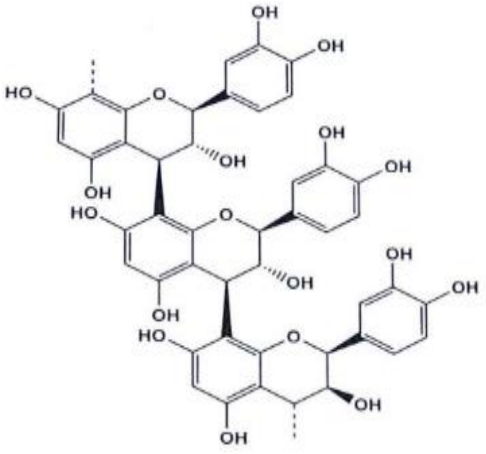
Table 3: Some plant-based Steroids with their respective plant name, family, mosquito species targeted, plant part used, LC_{50} value, and references

S.N.	Plant Name	Family	Mosquitospecies targeted	PlantPart used	LC50 Value	References
1	*Jatropha curcas*	Euphorbiaceae	*Aedes aegypti*	leaf	88 mg/mL	[19]
2	*C. papaya*	Caricaceae	*Cx. quinquefasciatus*	seed	0.13µg/ml	[40]
3	*M. paniculata*	Rutaceae	*Cx. quinquefasciatus*	Leaf	0.08 µg/ml	
4	*C. collinus*	Phyllanthaceae	*Cx. quinquefasciatus*	leaf	0.09 µg/ml	
5	*Solanumvillosum*	Solanaceae	*Ae. aegypti*	berry	60 to 538 ppm	[41]
6	*Artemisia absinthium*	Asteraceae	*Aedes aegypti*	flower	694.3 ppm	[42]
7	*Indigofera arrecta*	Fabaceae	*Culex mosquito.*	leaf	2752.6 ppm	[42]
8	*Elytraria acaulis*	Acanthaceae	*Aedes aegypti*	leaf	124.25 mg/100ml	[43]
9	*Dregea volubilis*	Apocynaceae	*Cx. quinquefasciatus*	leaf	31.29 ppm	[44]
10	*Bombax malabaricum*	Bombacaceae	*Cx. quinquefasciatus*	leaf	23.55 ppm	

Tannins

Tannins are chemical compounds commonly found in various plant species. They have been explored for their potential as mosquito larvicides due to their insecticidal properties. The typical structures of hydrolyzable and condensed tannins are presented in Figure 5. Mosquito larvicides are substances used to control mosquito larvae in their aquatic breeding habitats, preventing them from maturing into disease-spreading adults. The efficacy of tannins as mosquito larvicides can vary based on several factors, including the tannin concentration, mosquito species, environmental conditions, and the presence of other organic matter in the water. Potential modes of action include disrupting the mosquito digestive system, interfering with nutrient absorption, and affecting the development of mosquito larvae [45]. Table 4 lists some plant-based tannins, their respective plant names and families, and their effectiveness as mosquito larvicides.





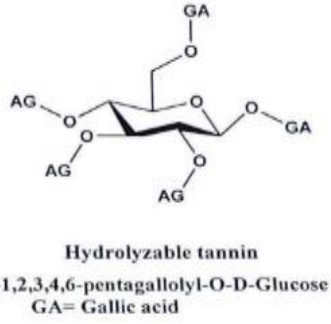


Fig 5: Typical Structures of hydrolyzable and condensed tannins.

Table 4: Some plant-based tannins along with their respective plant name, family, mosquitospecies targeted, plant part used, LC_{50} value, and references

SN	Plant Name	Family	Mosquito speciestargeted	Plant partused	LC_{50} value(mg/L)	References
1	*Rumex vesicarius*	Polygonaceae	*Aedes aegypti*	flower	19.99mg/l	[46]
2	*Prunus domestic*	Rosaceae	*Culex pipiens*	leaves	33.3mg/l	[47]
3	*Rhamnus cathartica*	Rhamnaceae	*Culex pipiens*	leaves	63.4 mg/l	
4	*Cassia fistula*	Fabaceae	*Culex Quinquefasciatus*	bark	50.27 mg/l	[48]
5	*Nicotiana tabacum*	Solanaceae	*Culex quinquefasciatus*	flower	17.77 mg/l	
6	*Reichardiatingitana*	Asteraceae	*Aedes aegypti*	flower	46.85 mg/l	[46]
7	*Argemone mexicana*	Papaveraceae	*Culex quinquefasciatus*	flower	18.61ppm	[47]
8	*Saracaindica*	Fabaceae	*Cx. quinquefasciatus*	leaves	228.9ppm	[48]
9	*Clitoria ternatea*	Fabaceae	*An. stephensi*	leaves	65.2ppm	
10	*Clitoria ternatea*	Fabaceae	*Ae. aegypti*	roots	154.5ppm	

Glycosides:

Glycosides are a diverse group of natural compounds found in many plants. They are characterized by their chemical structure, which consists of a sugar molecule (glycone) attached to a non-sugar molecule (aglycone) via a glycosidic bond. Plants produce glycosides for various reasons, including defense against herbivores and pathogens. The ideal structure of glycosides is depicted in Figure 6 below.

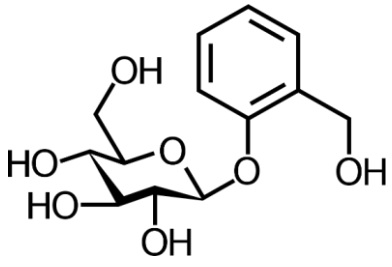


Fig 6: Structure of Glycosides

Glycosides may exert neurotoxic effects on mosquito larvae by impacting their nervous system, leading to paralysis and death. Certain glycosides can disrupt cellular processes and result in cell death in mosquito larvae [51].

Plant families known for the presence of glycosides include Araceae, Asteraceae, Gramineae, and Fabaceae - all of which have been reported for mosquitocidal activities (Table 5).

Table 5: Some plant-based glycosides along with their respective plant name, family, mosquito species targeted, plant part used, LC50 value, and references

SN	Name	Family	Mosquito species targeted	Plant part used	LC_{50} value (ppm)	References
1.	*Typhonium trilobatum*	Araceae	*Cx. quinq uefasciatus*	leaf	19.87 ppm	[52]
2.	*Cassia mimosoides*	Fabaceae	*Anophelesgambiae*	leaf	0.28 mg/ml	[51]
3.	*Duranta erecta*	Verbenaceae	*Anopheles gambiae*	leaf	10.037 ppm	[53]
4.	*Tridax procumbens*	Asteraceae	*Anopheles gambiae*	leaf	213.410 ppm	
5.	*Tridax procumbens*	Gramineae	*Anopheles gambiae*	leaf	214.417 ppm	
6.	*Ageratumconyzoide*	Asteraceae	*Anophelesgambiae*	leaf	423.520 ppm	[54]
7.	*Annona squamosa*	Annonaceae	*An. subpictus*	bark	93.80 mg/l	[55]
8.	*Chrysanthemum indicum*	Asteraceae	*Cx. tritaeniorhynchus*	leaf	39.98 mg/l	
9.	*Tridax* procumbens	Asteraceae	*Cx. tritaeniorhynchus*	leaf	51.57 mg/l	
10	*Stemona curtisii*	Stemonaceae	*Ae. aegypti*	aerial parts	358 mg/mL	[56]

Saponins:

Saponins are a class of naturally occurring compounds found in various plants, including some medicinal herbs. Known for their diverse biological activities, they have been extensively studied for potential applications in different fields, such as medicine and agriculture, due to their insecticidal properties. Given their amphipathic nature, saponins exhibit surfactant action, which may allow them to interact with phospholipids and cholesterol in cell membranes. This characteristic could potentially inform the development of medications and cosmetics. As a subclass of the larger terpenoid class of metabolites, saponins hold a specific place in plant biochemistry. Figure 7 depicts their basic structure.

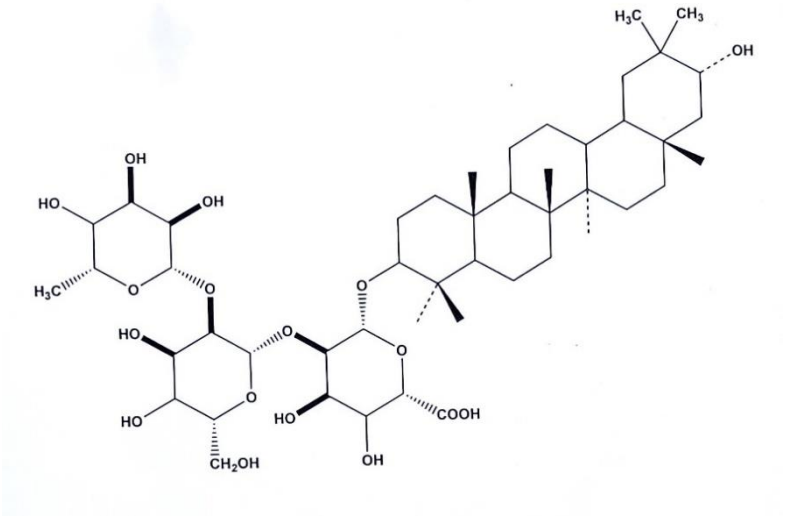


Fig 7: Basic structure of saponins

The larvicidal action of saponins involves disrupting the cell membranes of mosquito larvae, leading to cell lysis and ultimately causing their death [57]. Table 6 lists several plant-based saponins effective against different vector mosquitoes.

Table 6: Some plant-based Saponins along with their respective plant name, family, mosquitospecies targeted, plant part used, LC_{50} value, and references

S.N	Plant Name	Family	Mosquito species targeted	Plantpart used	LC_{50} value(ppm)	References
1	*Sapindusmukorossi*	Sapindaceae	*Aedes aegypti*	fruits	10.05 ppm	[58]
2	*Achyranthesaspera*	Amaranthaae	*Ae. aegypti*	leaf	18.20 ppm	[59]
3	*Tagetes minuta*	Asteraceae	*Anopheles stephensi*	flower	2.5 mg/l	[60]
4	*Gymnema sylvestre*	Apocynaceae	*Culex tritaeniorynchus*	leaves	34.75 mg/ml	[61]
5	*Solanum lycocarpum*	Solanaceae	*Culex quinquefasciatus*	fruit	75.13 mg/l	[62]
6	*Rhizophora mangle*	Rhizophoraceae	*Anopheles gambiae*	leaves	225 ppm	[63]
7	*Rhizophora racemosa*	Rhizophoraceae	*Anopheles gambiae*	leaves	175 ppm	
8	*Schinopsisbrasiliensis*	Anacardiaceae	*Aedes aegypti*	stem	313 µg/ml	[64]
9	*Annona muricata*	Annonaceae	*Anopheles gambiae*	leaf	36.64 mg/ml	[65]
10	*Lawsonia inermis*	Lythraceae	*Anopheles stephensi*	leaves	69.40 ppm	[66]

Phenols:

Phenols are a class of organic compounds widely distributed in the plant kingdom and known for their diverse chemical structures, as shown in Figure 8. Many phenolic compounds, identified in various plant species, exhibit mosquito larvicidal properties. Mosquito larvicides are substances capable of effectively killing mosquito larvae, an essential approach to controlling mosquito populations and reducing the spread of mosquito-borne diseases. Phenols may interfere with the crucial enzymatic processes within the mosquito larvae, disrupting their metabolic pathways and leading to death [67]. Plant families known for being rich sources of phenols include Rosaceae, Lamiaceae, Asteraceae, Fabaceae, Rutaceae, among others. Some of these, listed in Table 7, have proven effective against immature stages of mosquitoes.

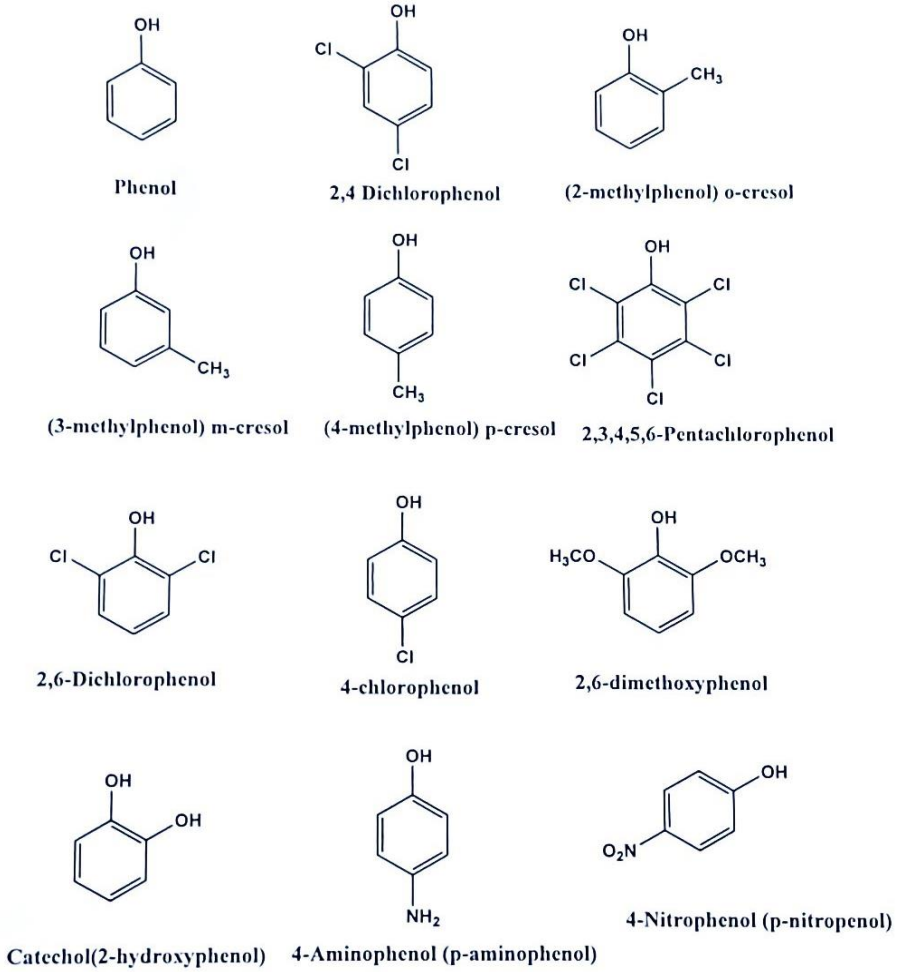


Figure 8: Structure of phenols

Table 7: Some plant-based Phenols along with their respective plant name, family, mosquito species targeted, plant part used, LC_{50} value, and references

S.N.	Plant Name	Family	Mosquito species targeted	Plant part used	LC_{50} value (ppm)	References
1.	*Anacardium occidentale*	Anacardiaceae	*Aedes aegypti and Culex quinquefasciatus*	leaves	5.4–22.6 mg/L	[68]
2.	*Kotschya thymodora*	Fabaceae	*Anopheles gambiae*	leaves	77.35 µg/mL	[69]
3.	*Salvia elegans*	Lamiaceae	*An. albopictus*	Aerial parts	46.4 ppm	[70]
4.	*Salvia splendens*	Lamiaceae	*Aedes albopictus*	Aerial parts	59.2 ppm	
5.	*Ricinus communis*	Euphorbiaceae	*An. stephensi*	leaves	0.75 ± 0.01 ppm	[71]
6.	*Callistemon rigidus*	Myrtaceae	*Cx. quinquefasciatus*	leaves	17.11 ppm	[72]
7.	*Eclipta prostrata*	Asteraceae	Cx. quinquefasciatus	Leaves	27.49 mg/l	[73]
8.	*Solanum trilobatum*	Solanaceae	*Cx. quinquefasciatus*	Leaf	265.69 ppm	[74]
9.	*Syzygium aromaticum*	Myrtaceae	*Ae. aegypti*	leaf	92.56 mg/l	[75]
10.	*Millingtonia hortensis*	Bignoniaceae	*Anopheles stephensi*	leaves	123 ppm	[76]

Essential oils

Essential oils have been studied for their potential use as mosquito larvicides due to their natural properties and the presence of certain phytochemicals. Phytochemicals are compounds derived from plants that often exhibit biological activity. Some essential oils have demonstrated larvicidal properties, as detailed in Table 8.

Compounds found in essential oils may induce oxidative stress within mosquito larvae, damaging their cells and tissues. Certain volatile compounds in essential oils may affect the respiratory processes of mosquito larvae, leading to suffocation and mortality [77].

Essential oils are prevalent in various plant families, and different plants within each family may yield essential oils with unique chemical compositions (Figure 9) and properties. Some plant families, famous for their essential oil production, include Apiaceae, Lauraceae, Pinaceae, Lamiaceae, and Myrtaceae.

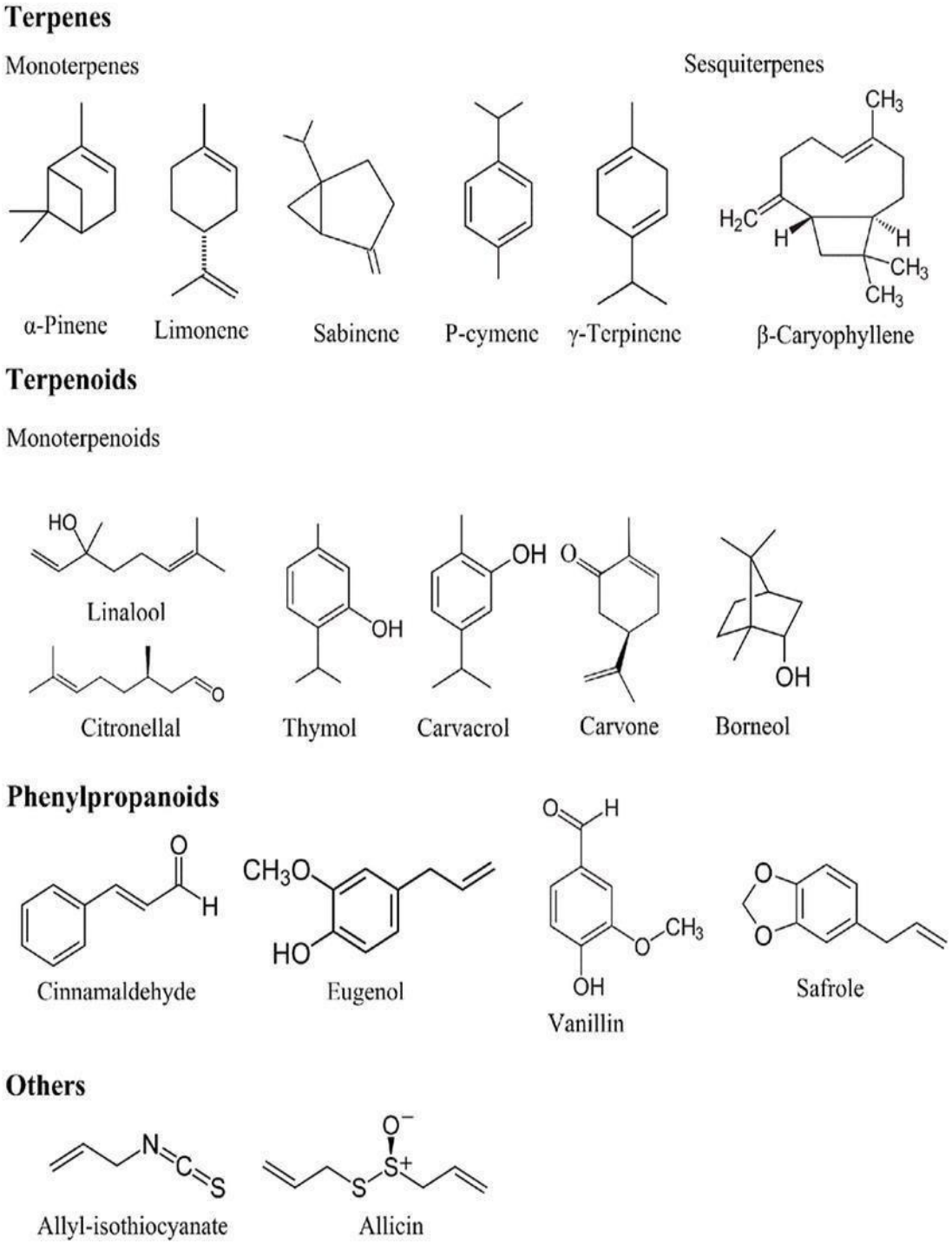


Fig 9: Structures of Essential oils

Table 8: Some plant-based Essential oils along with their respective plant name, family, mosquito species targeted, plant part used, LC_{50} value, and references

S.N.	Plant Name (Scientific Name)	Family	Mosquito species targeted	Plant part used	LC_{50} value (µg/mL)	References
1	*Tagetes patula*	Asteraceae	*Aedes aegypti*	flower	13.57 ppm	[78]
2	*Satureja bachtiarica*	Lamiaceae	*An. stephensi*	Leaves	24.27 ppm	[79]
3	*Satureja bachtiarica*	Lamiaceae	*Cx. quinquefasciatus*	leaves	44.96 ppm	
4	*Zingiber collinsii*	Zingiberaceae	*Ae. albopictus*	leaves	25.51 µg/mL	[80]
5	*Mentha spicata*	Lamiaceae	*Cx. quinquefasciatus*	leaf	62.62 ppm	[81]
6	*Ocimum americanum*	Lamiaceae	*A. aegypti*	*Leaves*	67 ppm	[82]
7	*Ocimum gratissimum*	Lamiaceae	*A. aegypti*	*Leaves*	60 ppm	
8	*Cinnamomum cassia*	Lauraceae	*A. aegypti*	Leaves	36 ppm	[83]
9	*Eucalyptus citriodora*	Myrtaceae	*Aedes aegypti*	Leaves	104.4 ppm	[84]
10	*Cymbopogon citratus*	Poaceae	*Aedes aegypti*	leaves	120.6 ppm	

Growth and reproduction inhibiting phytochemicals

Plant-based insecticides have the potential to significantly reduce the spread of vector-borne diseases by acting as repellents or insecticides. Plants primarily store phytochemicals in the form of secondary metabolites, facilitating the plant's defense mechanisms. Secondary metabolites such as alkaloids, steroids, terpenoids, tannins, and flavonoids, derived from various plants, have been reported above for their insecticidal properties. These phytochemicals are extracted from the entire body of small herbs or different parts of larger trees, including the fruits, leaves, stems, bark, and roots. In addition to serving as larvicides, these plant-derived phytochemicals can also be used as repellents, oviposition attractants, insect growth hormone regulators, and deterrents. Many regions globally have utilized plant products, either as extracts or whole plants, to either kill or repel mosquitoes [85].

Certain phytochemicals have a broad toxicant (insecticide/larvicide) effect on both mosquito larvae and adults. Others, known as growth inhibitors or chemosterilants, work by interfering with growth and development or reproduction, or they provide olfactory sensations that either attract or repel insects [86,87]. Factors such as the plant species, their geographic distribution, the extraction process, and the polarity of the solvent used during extraction all influence the insecticidal qualities of the phytochemicals. For mosquito control programs, extracts containing the active ingredient responsible for their mosquitocidal effect are extracted and utilized.

However, despite possessing favourable LC50 values, the majority of phytochemicals are ineffective due to the modest dose-response slope identified for most of them over a 24-hour period. Therefore, a phytochemical's ability to inhibit development may be crucial to the adoption of the substance by mosquito control businesses. The

judicious use of extraordinary phytochemicals could not only result in novel strategies but also prevent the emergence of insect resistance [88].

Scope for future research: isolation of toxic larvicidal active ingredients

Multiple research studies have demonstrated the effectiveness of plant extracts as a reservoir of bioactive toxic compounds against mosquito larvae. However, few have been extensively used in vector control regimens and commercially produced. Factors such as poor characterization and inefficiency in determining the structural configuration of active toxic components responsible for larvicidal action have contributed to the unsuccessful transition of bioactive toxic phytochemical discoveries from laboratory to practical applications. Any research design involving phytochemicals could consider the following stages for the manufacture of a green biopesticide [89,90,91]:

- Perform screening of floral biodiversity for crude plant extracts with potential to kill mosquito larvae;
- Prepare plant solvent extracts, starting with non-polar compounds and progressing to polar chemicals to identify the most effective solvent extract;
- Conduct a phytochemical analysis of the solid residue and apply column chromatography and thin-layer chromatography to purify and isolate the toxic phytochemical with larvicidal potential;
- Determine the lethal concentration (LC50/LC100 values);
- Evaporate the liquid solvent to obtain solid residue;
- Perform a phytochemical analysis of the solid residue; and
- Identify the structure of the active principle using infrared (IR) and nuclear magnetic resonance (NMR).

CONCLUSION

In conclusion, controlling mosquito populations is crucial in preventing the spread of lethal diseases such as malaria, dengue fever, and the Zika virus. Traditional mosquito control methods come with several limitations, including potential environmental impacts and the development of insecticide resistance. The use of natural products, such as mosquito larvicides, has gained attention as an alternative approach to controlling mosquito populations. Derived from plants, animals, and microorganisms, natural products offer several advantages, including low toxicity, biodegradability, and a reduced risk of resistance development. Therefore, the use of natural products like mosquito larvicides could offer a sustainable and effective solution to mosquito control. Further research is needed to explore the potential of natural products in mosquito control and identify new natural products with mosquito larvicidal properties.

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Conflicts of interest/Competing interests

The authors declared that they have no conflicts of interest.

Fig 10: Solanum nigrum (Alkaloids extracts from fruits)

Fig 11: *Tagetes patula* (Flavonoids reported from the flowers)

Fig 12: *Solanum villosum* (Steroid reported from berry)

Fig 13: *Clitoria ternatea* tannins reported from leaves and roots

Fig 14: *Cassia mimosoides* (Glycosides from leaf)

Fig 15: Saponnins reported from flowers of *Tagetes minuta*

Fig 16: *Salvia elegans* (Phenols from aerial parts)

Fig 17: *Ocimum americanum* (essential oil reported from leaves)

REFERENCES

1. Murthy U.S., Jamil K. Effect of the South Indian vetiver oil (*Vetiveria zizinioides* (L) Nash) against the immatures of *Culex quinquefasciatus* (Diptera: Culicidae) Say. *International pest control*. 1987; 29(1):8–9.
2. Kumar S., Sahgal A., Kumar S., Sahgal A. Advances in Mosquito Control: A Comprehensive Review. *Advances in Diptera - Insight, Challenges and Management Tools*. 2022, Doi: 10.5772/INTECHOPEN.106378.
3. Shaalan E.A.S., Canyon D., Younes M.W.F., Abdel-Wahab H., Mansour A.H. A review of botanical phytochemicals with mosquitocidal potential. *Environ Int*. 2005; 31(8):1149–1166. Doi: 10.1016/J.ENVINT.2005.03.003.
4. Ghosh A., Chowdhury N., Chandra G. Plant extracts as potential mosquito larvicides. *Indian J Med Res*. 2012; 135(5):581.
5. Ranson H., Lissenden N. Insecticide Resistance in African Anopheles Mosquitoes: A Worsening Situationthat Needs Urgent Action to Maintain Malaria Control. *Trends Parasitol*. 2016; 32(3):187–196. Doi:10.1016/j.pt.2015.11.010.
6. Krezanoski P., Haberer J. Objective monitoring of mosquito bednet usage and the ethical challenge of respecting study bystanders' privacy. 2019; 16(5):466–468. Doi:10.1177/1740774519865525.
7. Benelli G., Jeffries C.L., Walker T. Biological Control of Mosquito Vectors: Past, Present, and Future. *Insects*. 2016; 7(4):52. Doi: 10.3390/INSECTS7040052.
8. Engdahl C.S., Tikhe C.V., Dimopoulos G. Discovery of novel natural products for mosquito control. *Parasit Vectors*. 2022; (15)1:1–11. Doi:10.1186/S13071-022-05594-Z/FIGURES/5.
9. Mandal S. Mosquito vector management with botanicals-the most effective weapons in controlling mosquito-borne diseases. *Asian Pac J Trop Biomed*. 2012; 2(4): 336. Doi: 10.1016/S2221-1691(12)60035-5.
10. Ganesan P., Ignacimuthu S., Ganesan P., Ignacimuthu S. Metabolites from Actinobacteria for Mosquito Control. *Actinobacteria - Diversity, Applications and Medical Aspects*. 2022. Doi: 10.5772/INTECHOPEN.106885.
11. D. Varijakzhan *et al.* Bioactive Compounds from Marine Sponges: Fundamentals and Applications. *Marine Drugs*. 2021; 19(5):246. Doi:10.3390/MD19050246.
12. Varijakzhan D., Loh J-Y., Yap W-S., Yusoff K., Seboussi R., Lim S-H.E., Lai K-S., Chong C-M. Bioactive Compounds from Marine Sponges: Fundamentals and Applications. *Mar. Drugs*. 2021; 19:246. https://doi.org/10.3390/md19050246
13. Govindarajan M., Hoti S.L., Rajeswary M., Benelli G. One-step synthesis of polydispersed silver nanocrystals using *Malva sylvestris*: an eco-friendly mosquito larvicide with negligible impact on non-target aquatic organisms. *Parasitol Res*. 2016; 115(7):2685–2695. Doi: 10.1007/S00436-016-5038-X/TABLES/5.
14. Luker H.A., Salas K.R., Esmaeili D., Holguin F.O., Bendzus-Mendoza H., Hansen I.A. Repellent efficacy of 20 essential oils on *Aedes aegypti* mosquitoes and Ixodes scapularis ticks in contact-repellency assays. *Scientific Reports*. 2023; 13(1):1–10. Doi:10.1038/s41598-023-28820-9.
15. Britannica, The Editors of Encyclopaedia. "alkaloid". Encyclopedia Britannica, 21 May. 2023, https://www.britannica.com/science/alkaloid. Accessed 18 October 2023.
16. Mohanty S.S., Prakash S. Effects of culture media on the larvicidal property of secondary metabolites of mosquito pathogenic fungus *Chrysosporium lobatum* (Moniliales: Moniliaceae). *Acta Trop*. 2009; 109(1):50–54. Doi: 10.1016/J.ACTATROPICA.2008.09.013.

17. Kim S.I., Ahn Y.J. Larvicidal activity of lignans and alkaloid identified in *Zanthoxylum piperitum* barktoward insecticide-susceptible and wild *Culex pipiens pallens* and *Aedes aegypti*. *Parasit Vectors*. 2017; 10(1):1–10. Doi: 10.1186/S13071-017-2154-0/TABLES/5.
18. Oliveros-Díaz A.F., Pájaro-González Y., Cabrera-Barraza J., Catherine Hill C., Quiñones-Fletcher W., Olivero-Verbel J., Castillo F.D. Larvicidal activity of plant extracts from Colombian North Coast against Aedesaegypti L. mosquito larvae. *Arabian Journal of Chemistry*. 2022; 15(12):104365. Doi: 10.1016/J.ARABJC.2022.104365.
19. Malathi P., Vasugim S.R. Evaluation of mosquito larvicidal effect of Carica Papaya against Aedes Aegypti. *International Journal of Mosquito Research*. 2015; 2(3):21–24. Doi: 10.2149/tmh1973.28.177.
20. Gutierrez P.M., Antepuesto A.N., Eugenio A.L., Fleurellei M., Santos L., Larvicidal Activity of SelectedPlant Extracts against the Dengue vector Aedes aegypti Mosquito. *International Research Journal of Biological Sciences*. 2014; 3(4):23–32.
21. Sukari M.A., Noor H.M., Bakar N.A., Ismail S., Rahmani M., Abdul A.B. Larvicidal carbazole alkaloids from *Murraya koenigii* against dengue fever mosquito *Aedes aegypti* Linnaeus. *Asian Journal of Chemistry*. 2013, 25(14): 7719.
22. Rajasekaran A., Duraikannan G. Larvicidal activity of plant extracts on Aedes Aegypti L, *Asian Pac J Trop Biomed*. 2012; 2(3). Doi:10.1016/S2221-1691(12)60456-0.
23. Hadjiakhoondi A., Vatandoost H., Khanavi M., Abaee M.R., Karami M. Biochemical Investigation ofDifferent Extracts and Larvicidal Activity of *Tagetes minuta* L. on *Anopheles stephensi* Larvae. *Iranian Journal of Pharmaceutical Sciences*. 2005; 1(2):81–84.
24. Bouabida H., Dris D. Phytochemical constituents and larvicidal activity of Ruta *graveolens*, *Ruta montana* and *Artemisia absinthium* hydro-methanolic extract against mosquito vectors of. *Elsevier*. 2023.
25. Hemalatha P., Elumalai D., Janaki A., Babu M., Velu K., Velayutham K., Kaleena P.K. Larvicidal activity of *Lantana camara* aculeata against three important mosquito species. *Journal of entomology and Zoology Studies*. 2015; 3(1):174–181.
26. Rawani A., Chowdhury N., Ghosh A., Laskar S., Chandra G. Mosquito larvicidal activity of *Solanum nigrum* berry extracts. *Indian J Med Res*. 2013; 137(5):972-6. PMID: 23760385; PMCID: PMC3734691.
27. Inaba K., Ebihara K., Senda M., Yoshino R., Sakuma C., Koiwai K., Takaya D., Watanabe C., Watanabe A., Kawashima Y., Fukuzawa K., Imamura R., Kojima H., Okabe T., Uemura N., Kasai S., Kanuka H., Nishimura T., Watanabe K., Inoue H., Fujikawa Y., Honma T., Hirokawa T., Senda T., Niwa R. Molecular action of larvicidal flavonoids on ecdysteroidogenic glutathione S-transferase Noppera-bo in *Aedes aegypti*. BMC Biol. 2022; 20(1):43. Doi: 10.1186/s12915-022-01233-2. PMID: 35172816; PMCID: PMC8851771.
28. Gautam K., Kumar P., Poonia S., Larvicidal activity and GC-MS analysis of flavonoids of *Vitex negundo* and *Andrographis paniculata* against two vector mosquitoes *Anopheles stephensi* and *Aedes aegypti*. *J Vector Borne Dis*. 2013; 50(3):171-8. PMID: 24220075.
29. Rajkumar S., Jebanesan A. Bioactivity of flavonoid compounds from *Poncirus trifoliata* L. (Family: Rutaceae) against the dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). *Parasitol Res.* 2008; 104(1):19–25. Doi: 10.1007/S00436-008-1145-7.
30. C. A. Granados-Echegoyen *et al*. *Argemone mexicana* (Papaverales: Papavaraceae) as an Alternative mosquito Control: First Report of Larvicidal Activity of Flower Extract. *J Med Entomol*. 2019; 56(1):261–267. Doi: 10.1093/JME/TJY159.
31. Patel H.V., Patel J.D., Patel B. Comparative efficacy of phytochemical analysis and antioxidant activity of methanolic extract of *Calotropis gigantea* and *Calotropis procera*. Research gate. *Int J Life Sci Bio technol Pharm Res*. 2014, Aug. 21, 2023.

32. Swargiary A., Brahma K., Boro T., Daimari M., Roy M.K. Study of phytochemical content, antioxidant and larvicidal property of different solvent extracts of *Clerodendrum infortunatum* and *Citrus grandis*. *Indian Journal of Traditional Knowledge*. 2021; 20(2):329–334.
33. H. Bouabida, Dris D. Phytochemical constituents and larvicidal activity of Ruta *graveolens*, *Ruta montana* and *Artemisia absinthium* hydro-methanolic extract against mosquito vectors of. *Elsevier*. 2022. 151(B):504-511. https://doi.org/10.1016/j.sajb.2022.05.017
34. Dhandapani A., Kadarkarai M. HPTLC Quantification of Flavonoids, Larvicidal and Smoke Repellent Activities of *Cassia occidentalis* L. (Caesalpiniaceae) against Malarial Vectore *Anopheles Stephensi* Lis (Diptera: Culicidae). *Journal of Phytology*. 2011; 3(2).
35. Vimala R.T.V., Sathishkumar G., Sivaramakrishnan S. Optimization of reaction conditions to fabricate nano-silver using *Couroupita guianensis* Aubl. (leaf & fruit) and its enhanced larvicidal effect. *Spectrochim Acta A Mol Biomol Spectrosc*. 2015; 135:110-5. Doi: 10.1016/j.saa.2014.06.009. Epub 2014 Jun 27. PMID: 25062056.
36. Munhoz V.M., Longhini R., Souza J.R.P., Zequic J.A.C., Mello E.V S. L., Lopes G.C., Melloa, J.C.P. Extraction of flavonoids from *Tagetes patula*: process optimization and screening for biological activity. *Rev Bras Farmacogn*. 24(2014); 576-583.
37. Zuharah W., Yousaf A., Ooi K.L., Sulaiman S.F. Larvicidal activities of family Anacardiaceae on Aedes mosquitoes (Diptera: Culicidae) and identification of phenolic compounds. *Journal of King Saud University – Science*. 2021; 33(5):101471.
Doi: 10.1016/j.jksus.2021.101471
38. Zheng R., Li S., Zhang X., Zhao C. Biological Activities of Some New Secondary Metabolites Isolated from Endophytic Fungi: A Review Study. *Int J Mol Sci*. 2021; 22(2):959. Doi: 10.3390/ijms22020959. PMID: 33478038; PMCID: PMC7835970.
39. Chen X., Li Y., Jiang L., Hu B., Wang L., An S., Zhang X. Uptake, accumulation, and translocation mechanisms of steroid estrogens in plants. *Sci Total Environ*. 2021; 753:141979. Doi: 10.1016/j.scitotenv.2020.141979. Epub 2020 Aug 25. PMID: 32890876.
40. Rawani A., Haldar K.M., Ghosh A., Chandra G. Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res. 2009; 105(5):1411-7. Doi: 10.1007/s00436-009-1573-z. Epub 2009 Jul 31. PMID: 19644705.
41. Chowdhury N., Laskar S., Chandra G. Mosquito larvicidal and antimicrobial activity of the protein of *Solanum villosum* leaves. *BMC Complement Altern Med*. 2008; 8:62. Doi: 10.1186/1472-6882-8-62. PMID: 19061512; PMCID: PMC2642758.
42. Sofi M.A., NandaA., Sofi M.A., Maduraiveeran R. Larvicidal activity of *Artemisia absinthium extracts* with special reference to inhibition of detoxifying enzymes in larvae of *Aedes aegypti*. *Elsevier, Journal of King Saud University – Science*. 34(3):102248,
Doi: 10.1016/j.jksus.2022.102248.
43. O.M.R. Neema., Francis R. The larvicidal activity of *Indigofera arrecta* leaf extract against culex mosquito larvae. *International Journal of Bioassays*. 2015, researchgate.net.
44. Sukumaran S. and Maheswaran R. Larvicidal Activity of *Elytraria acaulis* against *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *J Arthropod Borne Dis*. 2020; 14(3):293-301. Doi: 10.18502/jad. v14i3.4563. PMID: 33644243; PMCID: PMC7903361.
45. Hossain E., Rawani A., Chandra G., Mandal SC, Gupta J.K. Larvicidal activity of *Dregea volubilis* and *Bombax malabaricum* leaf extracts against the filarial vector *Culex quinquefasciatus*. *Asian Pac J Trop Med*. 2011; 4(6):436-41. Doi: 10.1016/S1995-7645(11)60121-1. PMID: 21771694.

46. Rajkumar S., Jebanesan A. Larvicidal and oviposition activity of *Cassia obtusifolia* Linn (Family: Leguminosae) leaf extract against malarial vector, *Anopheles stephensi Liston* (Diptera: Culicidae). *Parasitol Res*. 2009; 104(2):337–340. Doi: 10.1007/S00436-008-1197-8.
47. Salama S.A., Al-Faifi Z.E., Masood M.F., El-Amier Y.A. Investigation and Biological Assessment of*Rumex vesicarius* L. Extract: Characterization of the Chemical Components and Antioxidant, Antimicrobial,Cytotoxic, and Anti-Dengue Vector Activity. *Molecules.* 2022; 27(10):3177. Doi:10.3390/MOLECULES27103177.
48. Shehata A.Z.I. Biological activity of *Prunus domestica* (Rosaceae) and *Rhamnus cathartica* (Rhamnaceae) leaves extracts against the mosquito vector, *Culex pipiens* L. (Diptera). journals. ekb. Eg. Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest. 2019.
49. Hamid Z.U.A., Ijaz A., Mughal T.K., Zia K., Correspondence Z., Ullah. Larvicidal activity of medicinal plant extracts against *Culex quinquefasciatus* Say. (Culicidae, Diptera). *Int. J. Mosq. Res*. 2018, 5(2):47-51. Doi: 10.13140/RG.2.2.36501.78565.
50. Granados-Echegoyen C.A., Chan-Bacab M.J., Ortega-Morales B.O., Vásquez-López A., Lagunez-Rivera L., Diego-Nava F., Gaylarde C., *Argemone mexicana* (Papaverales: Papavaraceae) as an Alternative for Mosquito Control: First Report of Larvicidal Activity of Flower Extract. *Journal of Medical Entomology*. 2019; 56(1):261–267. https://doi.org/10.1093/jme/tjy159.
51. Mathew N., Anitha M.G., Bala T.S.L., Sivakumar S.M., Narmadha R., Kalyanasundaram M. Larvicidal activity of *Saraca indica*, *Nyctanthes arbor-tristis*, and *Clitoria ternatea* extracts against three mosquito vector species. *Parasitol Res.* 2009; 104(5):1017–1025. Doi: 10.1007/S00436-008-1284-X.
52. Alayo M.A., Femi-Oyewo M.N., Bakre L.G., Fashina A.O. Larvicidal potential and mosquito repellent activity of *Cassia mimosoides* extracts. *Southeast Asian J Trop Med Public Health*. 2015; 46(4):596-601. PMID: 26867378.
53. Haldar K.M., Ghosh P., Chandra G. Evaluation of target specific larvicidal activity of the leaf extract of *Typhonium trilobatum* against *Culex quinquefasciatus* Say. *Asian Pacific Journal of Tropical Biomedicine*. 2011; 1(2):S199-S203. Doi:10.1016/S2221-1691(11)60156-1.
54. Egunjobi F.B., Okoye I.C. Ovicidal and Larvicidal Activities of Ethanolic Leaf Extracts of Three Botanicals Against the Malaria Vector - *Anopheles gambiae*. *Int. Ann. Sci.* 2020; 9(1):111–121. Doi: 10.21467/ias.9.1.111-121.
55. Suwaiba H., Barde A.A., Mao P.S., Aliyu O.A. Larvicidal activity of *Ageratum conyzoides* L. extracts on *Anopheles gambiae* complex. gsconlinepress.com. 2018; 3:1–005. Doi: 10.30574/gscbps.2018.3.3.0027.
56. Krishnaveni K.V., ThaiyalNayaki R., Balasubramanian G.M. Effect of *Gliricidia sepium* leaves extracts on Aedes aegypti: Larvicidal activity. *Journal of Phytology*. 2015; 7:26-31. http://scienceflora.org/journals/index.php/jp/, Doi: 10.19071/jp. 2015. v7.2898.
57. Chalom S., Panyakaew J., Phaya M., Pyne, S.G., Mungkornasawakul P. Cytotoxic and larvicidal activities of Stemona alkaloids from the aerial parts and roots of *Stemona curtisii* Hook.f. *Nat Prod Res*. 2021; 35(22):4311–4316. Doi:10.1080/14786419.2019.1709188.
58. Nerio L.S., Olivero-Verbel J., Stashenko E. Repellent activity of essential oils: A review. *Bioresour Technol*. 2010; 101(1):372–378. Doi: 10.1016/J.BIORTECH.2009.07.048.
59. Jawale C.S. Larvicidal activity of some saponin containing plants against the dengue vector *Aedes aegypti*. *Trends Biotechnol Res*. 2014; 3(1).

60. Bagavan A., Rahuman A., Kamaraj C., Geetha K. Larvicidal activity of saponin from *Achyranthes aspera* against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research*. 2008. 103(1):223–229. Doi: 10.1007/s00436-008-0962-z.
61. Hadjiakhoondi A., Vatandoost H., Khanavi M., Abaee M.R., Karami M. Biochemical investigation of different extracts and larvicidal activity of *Tagetes minuta* L. on *Anopheles stephensi* larvae. *Iranian Journal of Pharmaceutical Sciences*, 2005, ijps. ir.
62. Elumalai K., Dhanasekaran S., Krishnappa K. Larvicidal activity of Saponin isolated from *Gymnema sylvestre* R. Br. (Asclepiadaceae) against Japanese Encephalitis vector, *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). *Eur Rev Med Pharmacol Sci*. 2013; 17(10):1404-10. PMID: 23740457.
63. Pereira T.M., Silva Vde C., Neto J.A.R., Alves S.N., Lima L.A.R.S. Larvicidal activity of the methanol extract and fractions of the green fruits of *Solanum lycocarpum* (Solanaceae) against the vector *Culex quinquefasciatus* (Diptera: Culicidae). *Rev Soc Bras Med Trop*. 2014; 47(5):646-8. Doi: 10.1590/0037-8682-0010-2014. PMID: 25467268.
64. Angaye T.C.N., Ohimain E.L., Siasia E.P., Asaigbe P.I., Finomo O.A. Larvicidal activities of the leaves of *Niger Delta* mangrove plants against *Anopheles gambiae*. *Sky Journal of Microbiology Research*. 2014; academia.edu.
65. Linhares L.P.M.B., Pereira B.V.N., Dantas M.K.G., Bezerra W.Md.S Viana-Marques Dd.A., de Lima L.R.A., Sette-de-Souza P.H., *Schinopsis brasiliensis* Engler—Phytochemical Properties, Biological Activities, and Ethnomedicinal Use: A Scoping Review. *Pharmaceuticals*. 2022; 15(8):1028. https://doi.org/10.3390/ph15081028.
66. Amakiri P.C., Nwankwo, E.N., Amakiri, A.C., IEgbuche C.M., Osuagwu I.F., Okwelogu I.S., Offor V.O., Acha C.T. Phytochemical analysis and toxicity of *Annona muricata* stem bark and leaf extracts on *Anopheles gambiae* larvae. Unpublished. 2019. https://doi.org/10.13140/rg.2.2.28329.13927.
67. Ghosh A., Chowdhury N., Chandra G. Plant extracts as potential mosquito larvicides. *Indian J Med Res*. 2012; 135(5):581-98. PMID: 22771587; PMCID: PMC3401688.
68. de Carvalho G.H.F., de Andrade M.A., de Araújo C.N., Santos M.L., de Castro N.A., Charneau S., Monnerat R., de Santana J.M., Bastos I.M.D. Larvicidal and pupicidal activities of eco-friendly phenolic lipid products from *Anacardium occidentale* nutshell against arbovirus vectors. *Environ Sci Pollut Res Int*. 2019; 26(6):5514-5523. Doi: 10.1007/s11356-018-3905-y. Epub 2019 Jan 4. PMID: 30610586.
69. Daniel J., Innocent E., Sempombe J., Mugoyela V., Fossen T. Isolation and characterization of larvicidal phenolic acids from *Kotschya thymodora* Leaves. *Journal of Applied Sciences and Environmental Management*. 2020; 24(8):1483–1488. Doi: 10.4314/jasem.v24i8.26.
70. Mathew J. and Thoppil J.E. Chemical composition and mosquito larvicidal activities of Salvia essential oils. *Pharmaceutical Biology*, 2011; 49(5):456-463, Doi:10.3109/13880209.2010.523427.
71. Soni N. and Dhiman R.C. Phytochemical, anti-oxidant, larvicidal, and antimicrobial activities of castor (*Ricinus communis*) synthesized silver nanoparticles. *Chinese Herbal Medicines Elsevier*. 2017; 9(3):289-294, Doi: 10.1016/S1674-6384(17)60106-0.
72. Pierre D.Y., Okechukwu E.C., Nchiwan N.E. Larvicidal and phytochemical properties of *Callistemon rigidus* R. Br. (Myrtaceae) leaf solvent extracts against three vector mosquitoes. *J Vector Borne Dis*. 2014; 51(3):216-23. PMID: 25253215.

73. Rajakumar G., Rahuman A.A. Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vectors. *Acta Trop*. 2011; 118(3):196-203. doi: 10.1016/j.actatropica.2011.03.003. Epub 2011 Mar 17. PMID: 21419749.
74. Lalitha A., Thangapandiyan S. Mosquito larvicidal efficacy of the acetone leaf extract of *Solanum trilobatum* against *Culex quinquefasciatus* and *Aedes aegypti*. *Asian Journal of Pharmaceutical and Clinical Research*. 2018; 11(12):273-6. Doi:10.22159/ajpcr. 2018.v11i12.27432.
75. Fayemiwo K.A., Adeleke M.A., Okoro O.P., Awojide S.H., Awoniyi I.O. Larvicidal efficacies and chemical composition of essential oils of *Pinus sylvestris* and *Syzygium aromaticum* against mosquitoes. *Asian Pac J Trop Biomed*. 2014; 4(1):30-4. Doi: 10.1016/S2221-1691(14)60204-5. PMID: 24144127; PMCID: PMC3819492.
76. Kaushik R. and Saini P. Screening of some semi-arid region plants for larvicidal activity against *Aedes aegypti* mosquitoes. *J Vector Borne Dis*. 2009; 46(3):244-6. PMID: 19724091.
77. Kelly P.H., Yingling A.V., Ahmed A., Hurwitz I., Ramalho-Ortigao M. Defining the mechanisms of action and mosquito larva midgut response to a yeast-encapsulated orange oil larvicide. *Parasit Vectors*. 2022; 15(1). Doi: 10.1186/S13071-022-05307-6.
78. Dharmagadda V.S., Naik S.N., Mittal P.K., Vasudevan P. Larvicidal activity of *Tagetes patula* essential oil against three mosquito species. *Bioresour Technol*. 2005; 96(11):1235-40. Doi: 10.1016/j.biortech.2004.10.020. Epub 2004 Dec 15. PMID: 15734310.
79. Moussa Soleimani-Ahmadi M., Abtahi S.M., Madani A., Paksa A., Abadi Y.S., Gorouhi M.A., Sanei-Dehkordi A. Phytochemical Profile and Mosquito Larvicidal Activity of the Essential Oil from Aerial Parts of *Satureja bachtiarica* Bunge Against Malaria and Lymphatic Filariasis Vectors. *Journal of Essential Oil-Bearing Plants*. 2017; (20)2:328–336. Doi: 10.1080/0972060X.2017.1305919.
80. Huong L.T., Huong T.T., Huong N.T.T., Hung N.H., Dat P.T.T., Luong N.X., Ogunwande I.A. Mosquito Larvicidal Activity of the Essential Oil of *Zingiber collinsii* against *Aedes albopictus* and *Culex quinquefasciatus*. *J Oleo Sci*. 2020; 69(2):153-160. Doi: 10.5650/jos. ess19175. PMID: 32023580.
81. Govindarajan M., Sivakumar R., Rajeswari M., Yogalakshmi K. Chemical composition and larvicidal activity of essential oil from *Mentha spicata* (Linn.) against three mosquito species. *Parasitol Res*. 2012; 110(5):2023-32. Doi: 10.1007/s00436-011-2731-7. Epub 2011 Dec 6. PMID: 22139403.
82. Cavalcanti E.S., Morais S.M., Lima M.A., Santana E.W. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. *Mem Inst Oswaldo Cruz*. 2004; 99(5):541-4. Doi: 10.1590/s0074-02762004000500015. Epub 2004 Nov 3. PMID: 15543421.
83. Cheng S.S., Liu J.Y., Tsai K.H., Chen W.J., Chang S.T. Chemical composition and mosquito larvicidal activity of essential oils from leaves of different *Cinnamomum osmophloeum* provenances. *J Agric Food Chem*. 2004; 52(14):4395-400. Doi: 10.1021/jf0497152. PMID: 15237942.
84. Manh H.D., Hue D.T., Hieu N.T.T., Tuyen D.T.T., Tuyet O.T. The Mosquito Larvicidal Activity of Essential Oils from Cymbopogon and Eucalyptus Species in Vietnam. *Insects*. 2020; 11(2):128. https://doi.org/10.3390/insects11020128.

85. Su T. and Mulla M.S. Antifeedancy of neem products containing Azadirachtin against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). *J Vector Ecol.* 1998; 23:114-122.
86. Babu R., Murugan K., Interactive effect of neem seed kernel and neem gum extracts on the control of *Culex quinquefasciatus* say. *Neem Newsletter*. 1998; 15(2):9-11.
87. Venkatachalam M.R., Jebanesan A. Repellent activity of *Ferronia elephantum* Corr. (Rutaceae) leaf extracts against *Aedes aegypti*. *Biores Technol*. 2001; 76:287-288.
88. Shaalan E.A.S., Canyon D., Younes M.W.F., Abdel-Wahab H., Mansour A.H. A review of botanical phytochemicals with mosquitocidal potential. *Environ Int*. 2005;31(8):1149-66.
Doi: 10.1016/j.envint.2005.03.003. PMID: 15964629.
89. Alsarhan, A., BaniSalman, K., Olimat, S. Chemical Composition of the Essential Oils of the Flowers *Asphodelus aestivus* Brot. Grown Wild in Jordan. *Jordan Jordan j. pharm. Sci*. 2023; 16(4):734-739.
DOI: https://doi.org/10.35516/jjps.v16i4.1082
90. Yabansabra, Y.R., Bebari, S.F., Simaremare, E.S. The Effectiveness of Zodia Leaves (*Evodia Suaveolens* Scheff) Oil as *Aedes aegypti* L Mosquito Repellent in Papua. *Jordan j. pharm. Sci*. 2023; 16(2):207-216.
DOI: https://doi.org/10.35516/jjps.v16i2.1321
91. Nour, S., Salama, M., Mahrous, E., El-Askary, H., Hifnawy, M., & Abdel Kawy, M. A. *Severinia buxifolia* Leaves: Isolation, Characterization of Major Metabolites from the Bioactive Fractions and their Antiprotozoal Activity. *Jordan j. pharm. Sci*. 2023; 16(1),18–29.
https://doi.org/10.35516/jjps.v16i1.1033

مراجعة للتطورات الحديثة للمنتجات الطبيعية كمبيدات لليرقات في إدارة مكافحة ناقلات الأمراض

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ملخص

البعوض، وهو ناقل بيولوجي، مسؤول عن نقل بعض الأمراض الخطيرة والمخيفة في العالم كله. هذه الأمراض مستوطنة في البلدان الاستوائية بشكل رئيسي وتتسبب في وفاة الملايين كل عام. أصبحت المبيدات الحشرية ذات الأصل النباتي والصديقة للبيئة أكثر أهمية في السنوات الأخيرة. ونظرًا إلى قابليتها في التحلل البيولوجي وانتقائية الأهداف، يمكن استخدامها بأمان في البيئات المائية. على الرغم من أن تطبيقات المبيدات الحشرية فعالة للغاية في السيطرة على أنواع النواقل المستهدفة، إلا أنها تسبب خطرًا لأن مقاومة المبيدات الحشرية الكيميائية آخذة في التزايد، مما يؤدي إلى انتعاش القدرة الناقلية. تستكشف هذه الورقة فعالية المواد الكيميائية النباتية في مكافحة مجموعات البعوض الناقل. وفي برامج مكافحة البعوض، يكون للمواد الكيميائية النباتية تأثيرا كبيرا. تعمل النباتات كمخزن ضخم للأيضات الأولية والثانوية. يمكن استخدام أنواع مختلفة من المذيبات القطبية وغير القطبية لاستخلاص المكونات النباتية النشطة بيولوجيًا من النبات بأكمله أو من جزء معين منه. تحدد هذه المراجعة الأدبية أيضًا المنتجات الطبيعية وتقدم نظرة عامة على الأنواع المختلفة من المنتجات الطبيعية التي يمكن استخدامها للسيطرة على يرقات البعوض. ويناقش بشكل خاص فعالية المنتجات الطبيعية في مكافحة ناقلات الأمراض دون التسبب في أي مقاومة أو ضرر للكائنات الحية الأخرى غير المستهدفة. الغرض من هذه الورقة هو تقديم نظرة شاملة عن استخدام المنتجات الطبيعية كمبيدات ليرقات البعوض وتسليط الضوء على إمكاناتها كبديل للطرق الكيميائية التقليدية. وهدف هذه الورقة هو تشجيع إجراء المزيد من البحوث في مجال تطوير واستخدام المنتجات الطبيعية لمكافحة البعوض الناقل للمرض بشكل فعال.

الكلمات الدالة: مكافحة ناقلة الأمراض، المنهج التقليدي، الإنتاجات الطبيعية، المواد الكيميائية النباتية.

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Evaluation of Anti-Inflammatory, Antioxidant Activities and Molecular Docking Analysis of *Rubus idaeus* Leaf Extract

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ABSTRACT

The study aimed to identify the most abundant compounds in raspberry leaf extract via HPLC analysis, conduct theoretical and practical assessments of antioxidant and anti-inflammatory activities both in silico, in vitro, and in vivo, and evaluate the correlation between antioxidant and anti-inflammatory activities. Polyphenols were quantified using HPLC; molecular docking was carried out using AutoDockTools 1.5.6; antioxidant activity was ascertained via the potentiometric method; and anti-inflammatory activity was examined based on the carrageenan edema method. The extract was found to be rich in epicatechin (0.417%), (+)-catechin (0.501%), and ellagitannins (0.401%). The free energy of (+)-catechin and epicatechin was -8.40 and -7.20 respectively for the active sites of cyclooxygenase-2 (COX-2), and -6.60 and -7.11 for nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase). Notably, the antioxidant activity of the raspberry leaf extract was 1.43%, 1.04%, and 10.62% higher than that of green tea leaf extract for doses of 4.00, 2.00, 0.20 mg/mL, respectively. Treatment with the raspberry leaf extract at a dose of 13.0 mg/kg resulted in a significant decrease in edema after 1, 2, and 3 hours by 38.8%, 41.8%, and 48.8%, respectively, compared to the control group. The study demonstrated a correspondence between experimental and theoretical results in evaluating antioxidant and anti-inflammatory activities. Correlation analysis further substantiated that the anti-inflammatory action is dependent on antioxidant activity.

Keywords: *Rubus idaeus* L., Leaf, HPLC, Molecular docking, Antioxidant activity, Anti-inflammatory activity, Correlation.

1. INTRODUCTION

In many chronic diseases such as diabetes mellitus, hypertension, atherosclerosis, Alzheimer's disease, and cancer [1], inflammation and oxidative stress invariably play pivotal roles. During the inflammatory response, neutrophils and macrophages generate substantial quantities of free radicals to combat and eliminate foreign invaders [2,3]. Furthermore, recent research has revealed that non-phagocytic cells, like interleukin-6 (IL-6), can also produce free radicals by expressing NADPH oxidase [4]. Importantly, oxidative stress does not only arise from inflammation, but can also provoke it. Studies have shown that hydrogen peroxide free radicals can initiate inflammation by activating transcriptional enzymes like nuclear factor of kB (Nf-kB), p38 mitogen activated protein kinase, and N-terminal c-Jun kinase [5, 6, 7]. These findings underscore the interconnected nature of inflammation and oxidative stress, as each of these enzymes has the potential to trigger the other. Consequently, it is crucial for new medications to possess both antioxidant and anti-inflammatory properties.

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The genus Rubus consists of around 700 species that usually occur in the temperate climate [8]. Raspberries, members of the rose family, are aggregate fruits commonly grown and consumed throughout Asia, Europe, and America. They are closely related to blackberries and other brambles or caneberries. Although many species and types of raspberries exist, red and black berries are the most common [9]. Recent research indicates that raspberry leaves and fruits are a rich source of flavonoid derivatives, represented by quercetin derivatives as well as phenolic acids, organic acids, and vitamin C [10]. Durgo et al. [11] have declared that ellagic acid is the main component among phenolic compounds. It is represented in three different forms: ellagitannins, where ellagic acid forms esters with a sugar; free ellagic acid; and ellagic acid as glycosides. Raspberry fruits, leaves, and blossoms have been used for medicinal purposes. Raspberry leaves are typically applied to treat gastrointestinal disorders, respiratory issues, heart problems, the flu, fever, and diabetes. The fruits have traditionally been used as cardioprotective, antitumor, anti-inflammatory, and antipyretic agents [12, 13]. Raspberry blossoms were used to create eye ointments or to treat stomach ailments [14].

Prominent scientific studies indexed in PubMed and Scopus have unveiled the robust anti-inflammatory and antioxidant effects of ellagic acid and epicatechin. Mansury et al. [15] studied the anti-inflammatory and antioxidant activity in a carrageenan-induced mouse paw edema model. The study's findings revealed that ellagic acid, administered systemically at doses ranging from 1 to 30 mg/kg, displayed a dose-dependent reduction in edema in the inflamed paws of rats. Moreover, ellagic acid treatment led to decreased serum levels of nitric oxide (NO) and prostaglandin E2 (PGE2). Additionally, the expression of endothelial NOS (eNOS) and cyclooxygenase-2 (COX-2) enzymes were suppressed, while the production of tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) in the inflammatory paw tissue was attenuated.

In a recent in vitro study by Yang et al. [16], the objective was to explore the impact of epicatechin on the production of pro-inflammatory mediators in RAW264.7 cells induced by lipopolysaccharide. The results of the analysis revealed that epicatechin effectively suppressed the expression of eNOS and COX-2, along with reducing the production of NO, PGE2, and pro-inflammatory cytokines IL-6, IL-1β, and TNF-α in RAW264.7 cells.

In our recent studies, we found that Rubus idaeus leaf extract has anti-inflammatory, antioxidant, and antimicrobial effects [17]. Currently, limited data is available concerning the correlation between antioxidant and anti-inflammatory activities of Rubus idaeus leaves. Taking into account the pharmacological action of biologically active substances (BAS) contained in red Rubus idaeus leaves, we hypothesized a correlation between the anti-inflammatory and antioxidant activities of the obtained Rubus idaeus leaf extract. Therefore, the aim of this investigation was to determine the main BAS using the HPLC method, conduct theoretical and practical studies to ascertain the antioxidant and anti-inflammatory activity in silico, in vitro, and in vivo of Rubus idaeus leaf extract, and to study the correlation of pharmacological actions.

2. MATERIALS AND METHODS

2.1 Plant material

The object of the study was the leaves of Rubus idaeus, which were collected from places of its native cultivation. The material was gathered in 2021, after the fruiting period, in the vicinity of the village of Ternova, Kharkiv region (50.193116162220264, 36.66935288403296). Green tea leaves from the Chun Mee cultivar were collected in Anhui province, China, during the months of March to May.

2.2 Reagents

Methanol and trifluoroacetic acid were purchased from Allchem. Sanguinin H-10 isomer 1, Lambertianin C, Sanguinin H-6, (+)-catechin, (-)-epicatechin, ellagic acid, cyanidin-3-O-glucoside, and quercetin-3-O-glucuronide were procured from the Sigma-Aldrich Company.

2.3 Extraction procedure

An exact mass of 10.0g of Rubus idaeus leaves were ground to a size of 1-2 mm. The extraction was carried out in 60% ethanol, at a raw material to solvent ratio of 1/20 (m/v), on a water bath at 80ºC with a reflux condenser for one hour. This process was performed twice. After cooling, the solutions were filtered and concentrated to a final volume of 20 mL using a rotary evaporator at 40ºC under vacuum conditions.

The green tea extract was obtained by 60% ethanol according to the procedure mentioned in our previous research [18].

2.4 Experimental animals

The study involved 56 male rats of the outbred white strain, weighing between 180 and 220 grams. These rats were sourced from the vivarium of the National University of Pharmacy (NUPh). Throughout the experiment, the rats were housed in macrolon boxes, with five animals in each box. Rats had unrestricted access to water and food, which were provided on a daily basis. The bedding was replaced on a three-day cycle. The rats were maintained under specific conditions, including a temperature of 22±2°C, relative humidity of 60±5%, and a daily light cycle consisting of 12 hours of light and 12 hours of darkness.

All procedures carried out during the study adhered to the guidelines set by the National Institute of Health for the care and use of laboratory animals, as well as the European Council Directive on 24 November 1986 for the Care and Use of Laboratory Animals (86/609/EEC). The study protocol was approved by the Local Ethics Committee.

2.5 HPLC method of analysis

The chromatographic separation was carried out on an Agilent Technology model 1100 chromatograph with a 150 mm × 2.1 mm ZORBAX-SB C-18 column with a granularity at a pore size of 3.5 μm. The elution flow rate was 0.25 mL/min. All determinations were undertaken at 45 ºC. The mobile phase binary solvent system consisted of solvent A (0.6% trifluoroacetic acid) and solvent B (70% methanol) [19]. All solvents utilized in the experiment underwent ultrasonic degassing and were subjected to 0.22 μm pore size membrane filtering. The sample injection volume was set at 2 μL, and detection occurred at wavelengths of 254, 280, and 350 nm. The mobile phase gradient used was linear and followed the profile given in Table 1. The concentrations of phenolic compounds in the extract were calculated from standard curves using the standard of individual compounds.

Table 1. Linear mobile phase gradient

Time, min	0.6% trifluoroacetic acid	70% methnol
0	92	8
8	62	38
24-29	0	100

Standard calibration

Stock solutions (2 mg/mL) for phenolic compounds were prepared by accurately weighing 50 mg of each substance into 25.0 mL of methanol. Dilution of the above stock solutions yielded a set of standard solutions of 200, 100, 50, and 25 μg/mL for each individual compound, respectively. Calibration curves were obtained for each individual compound by plotting concentrations versus peak areas. Regression equations were obtained from the calibration curves for each individual phenolic compound. Identification of the phenolic compounds was done by comparing the retention time of the unknown compounds with those of authentic phenolic compounds at three wavelengths (254, 280, 350 nm). The identities were then

confirmed by spiking the unknown samples with authentic compounds.

2.7 Molecular docking

A molecular docking study was conducted using a tool known as AutoDockTools 1.5.6 [20]. The preparation of the protein involved an optimization process, which included the removal of water and other atoms, followed by the addition of a polar hydrogen group. Autogrid was used to configure the grid coordinates (X, Y, and Z) on the binding site. Genetic algorithm parameters were applied for ligand interaction, with 10 runs of this criterion.

COX-2 (PDB ID: 1ddx) and NADPH oxidase (PDB ID: 5o0X) structures were obtained from the PDB database [21]. The resolution of 1ddx was 3.00 Å, whereas 5o0X was 2.20 Å. For the docking experiment, protein structure is selected if the resolution is above 2 Å. So, these two proteins can be used for the experiment. The ligand structures of (+)-catechin (CID_9064), (-)-epicatechin (CID_72276), and ellagic acid (CID_5281855) were obtained from the PubChem database [22]. The active site of the docking protein was identified utilizing the Computed Atlas for Surface Topography of Proteins (CASTp) [23].

2.8 Antioxidant activity

The extract's antioxidant activity was assessed using the potentiometric method [24]. Antioxidant activity was calculated according to the following equation and expressed as mmol-equiv./$m_{dry\ res.}$:

$$AOA = \frac{C_{OX} - \alpha \times C_{red}}{1+\alpha} \times K_{dil} \times 10^3 \times \frac{m_1}{m_2}$$

..where, $\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - E ethanol)nF/2.3RT}$; C_{ox} – concentration of $K_3[Fe(CN)_6]$, mol/l; C_{red} – concentration of $K_4[Fe(CN)_6]$, mol/l; $E_{ethanol} - 0.0546 \cdot C_{\%} - 0.0091$; $C_{\%}$ – concentration of ethanol; ΔE – change of potential; F = 96485.33 C/mol – Faraday constant; n = 1 – number of electrons in electrode reaction; R = 8.314 J/molK – universal gas constant; T – 298 K; K_{dil}– coefficient of dilution; m_1 – mass of dry residue; m_2 – mass of dry residue in 1.0 ml of extract.

2.9 Anti-inflammatory activity

The extract's anti-exudative activity was investigated using 56 male rats of the outbred white strain, weighing between 180 and 220 grams. The anti-inflammatory activity was carried out according to the carrageenan edema method [25]. The activity of the extract and reference drug were calculated using the following formula:

$$A = \frac{(M_s - M_h) \times 100}{M_{sh} - M_{hc}}$$

where A represents the anti-exudative activity (%), Ms is the volume of the swollen paw in the experiment, Mh is the volume of a healthy paw in the experiment, Msc is the volume of the swollen paw in the control, and Mhc is the volume of a healthy paw in the control.

The animals in the study were divided into six groups for experimental purposes. The first group served as the control group, where the animals were subplantarly administered a carrageenan solution and orally given 0.5 mL/kg of distilled water. The second, third, and fourth groups received the carrageenan solution subplantarly, along with intragastric administration of the studied extract at doses of 0.65 mg/kg, 6.0 mg/kg, and 13.0 mg/kg, respectively. Animals in the fifth group were given a comparison drug, specifically indomethacin at a dose of 2 mg/kg, intragastrically in addition to the carrageenan injection. The sixth group consisted of intact animals who received a subplantar administration of 0.1 mL saline solution.

2.10 Statistical analysis

The measurements were made five times (replicates). The results were expressed as mean values accompanied by standard deviations, reflecting the level of certainty in the measurements. Statistical analysis was performed using MS Excel 7.0 and STATISTIKA 6.0 software, enabling thorough data evaluation and interpretation.

3.RESULTS

3.1 HPLC analysis

The HPLC method was used to carry out a qualitative and quantitative analysis of phenolic compounds in the obtained extract of Rubus idaeus leaves. According to the results of the study, 15 compounds were identified (Fig. 1, 2, 3, and 4). The sum of polyphenols in the obtained extract was 1.680%, of which flavan-3-ols (catechins) accounted for 0.918% (54.64% of the total polyphenols), ellagitannins - 0.401% (23.87% of the total polyphenols), flavonols – 0.245% (14.58% of the total polyphenols), and ellagic acid derivatives - 0.113% (6.73% of the total polyphenols) (Table 2).

Among the flavan-3-ols, epicatechin dominates – 0.417±0.004% (24.82% of the total polyphenols), and (+)-catechin – 0.501±0.005% (29.82% of the total polyphenols). Among ellagitannins, 6 compounds were identified: Sanguiin H-10 isomer 1 - 0.026±0.001% (1.55% of the total polyphenols), Lambertianin C without ellagic acid fragment – 0.007±0.0001% (0.42% of the total polyphenols), Sanguiin H-10 isomer 2 - 0.024±0.001% (1.43% of the total polyphenols), Lambertianin C - 0.141±0.001% (8.39% of the total polyphenols), Sanguiin H-6 - 0.192±0.002% (11.43% of the total polyphenols), and Lambertianin C isomer – 0.011±0.001% (0.65% of the total polyphenols) (Table 2).

As shown in Table 1, Sanguiin H-6 dominates among all ellagitannins, Lambertianin C is in second place, and Sanguiin H-10 isomer 1 is in third place. The compound with the lowest content was Lambertianin C without the ellagic acid fragment. The content of ellagic acid was 0.068±0.004% (4.50% of the total phenolic compounds). As shown in the results, the content of ellagic acid and its derivatives is 72% lower than that of ellagitannins (Table 2).

Only one flavonol – quercetin-3-O-glucuronide – was identified in the Rubus idaeus leaves. The content of quercetin-3-O-glucuronide was 0.245±0.002% (14.58% of the total polyphenols). Moreover, one anthocyanin was identified – cyanidin-3-O-glucoside (0.003±0.001% (0.18% of the total polyphenols)), and its content is minor compared with other compounds (Table 2).

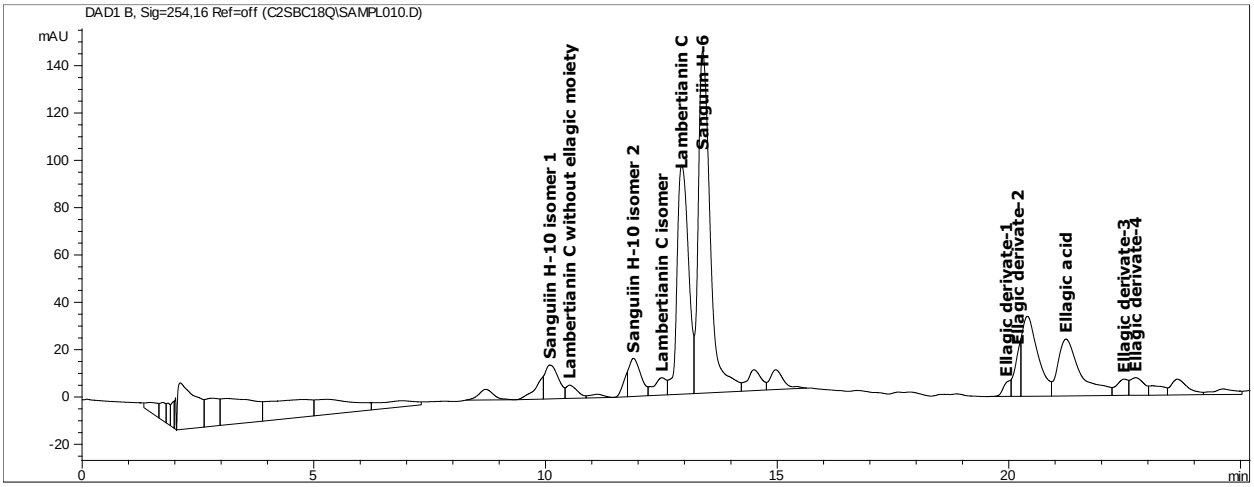

Figure 1. HPLC fingerprint (254 nm) of the *Rubus idaeous* leaves extract

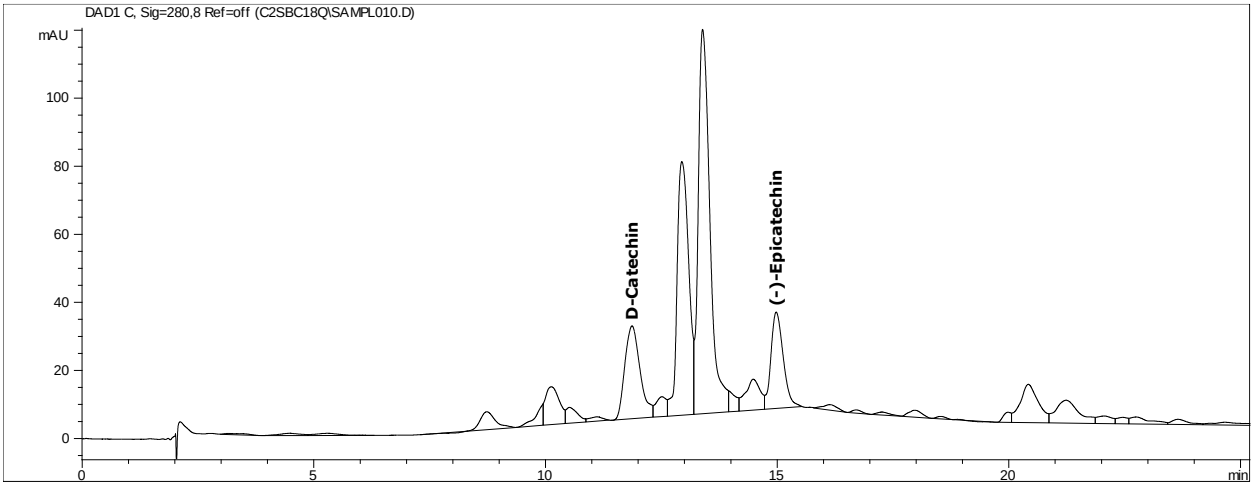

Figure 2. HPLC fingerprint (280 nm) of the *Rubus idaeous* leaves extract

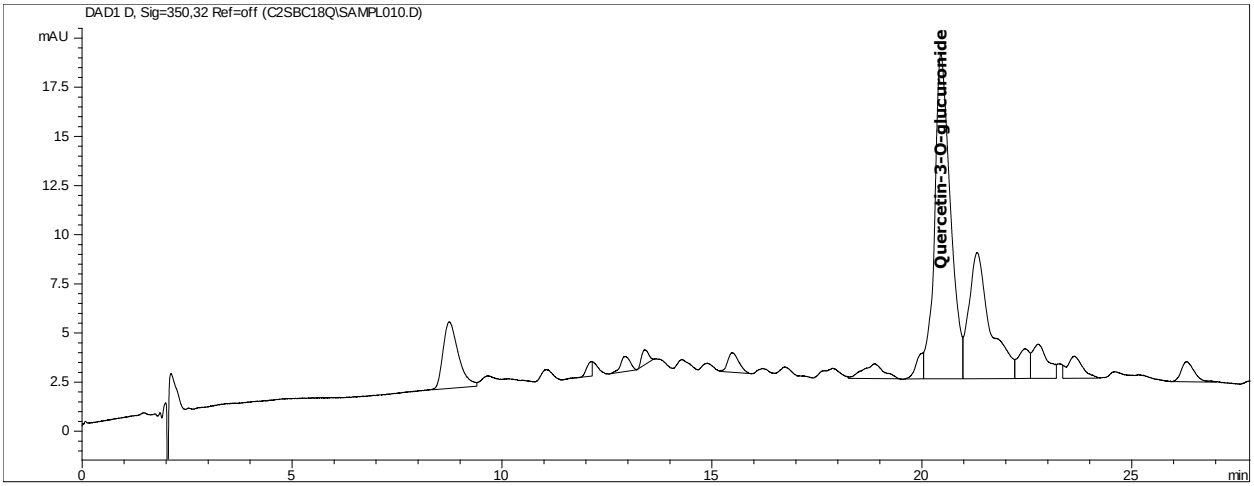

Figure 3. HPLC fingerprint (350 nm) of the *Rubus idaeous* leaves extract

Table 2. Qualitative composition and quantitative content of polyphenols in the extract of *Rubus idaeous* leaves

	Compound	Rt, min	Quantitative content, %	% out of sum polyphenols
1	Sanguiin H-10 isomer 1	10.08	0.026±0.001	1.55
2	Lambertianin C without ellagic fragment	10.51	0.007±0.0001	0.42
3	(+)-Catechin	11.89	0.501±0.005	29.82
4	Sanguiin H-10 isomer 2	11.91	0.024±0.001	1.43
5	Lambertianin C isomer	12.48	0.011±0.001	0.65
6	Lambertianin C	12.91	0.141±0.001	8.39
7	Sanguiin H-6	13.38	0.192±0.002	11.43
8	(-)-Epicatechin	14.96	0.417±0.004	24.82
9	Cyanidin-3-O-glucoside	18.43	0.003±0.001	0.18
10	Ellagic acid derivatives 1	19.96	0.006±0.001	0.36
11	Ellagic acid derivatives 2	20.26	0.016±0.001	0.95
12	Quercetin-3-O-glucuronide	20.44	0.245±0.002	14.58
13	Ellagic acid	21.20	0.068±0.001	4.05
14	Ellagic acid derivatives 3	22.48	0.010±0.001	0.60
15	Ellagic acid derivatives 4	22.75	0.013±0.001	0.77
	Total content of identified compounds		**1.680**	**100**

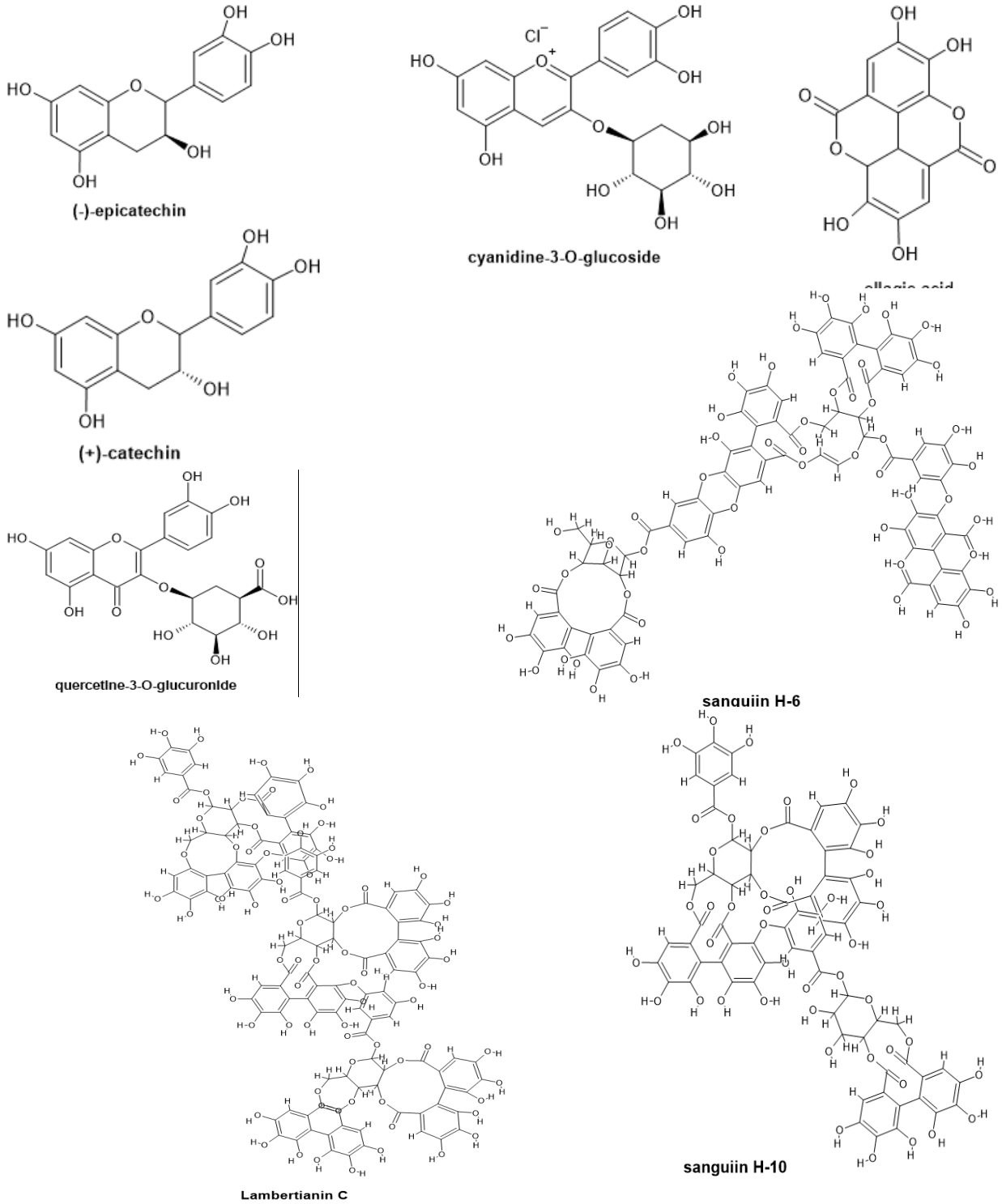

Figure 4. Structures of the identified phenolic compounds in the extract of *Rubus idaeous* leaves

3.2 Molecular docking

For the molecular modeling of theoretical antioxidant and anti-inflammatory activity, we selected (+)-catechin, epicatechin, and ellagic acid. Epicatechin and (+)-catechin were chosen because their content constituted 54.64% of all phenolic compounds in the obtained extract.

All studied compounds demonstrated a high level of affinity for the structure of the COX-2 enzyme. (+)-Catechin had the highest free energy value (-8.40 kcal/mol), followed by epicatechin (-7.94 kcal/mol). When comparing the results with the indometacin standard, the affinity of (+)-catechin with the COX-2 active site was 16% lower, and in the case of epicatechin, it was 21% lower than that of indometacin, respectively. Additionally, the theoretical dose of studied compounds required for 50% inhibition of the enzyme was calculated per kg of rat weight. Thus, the dose of (+)-catechin was 0.10 mg/kg, and for epicatechin it was 0.55 mg/kg. The dose of (+)-catechin was significantly higher than the dose of indomethacin by 17 times, and in the case of epicatechin, it was 92 times higher (Table 3).

The interaction of (+)-catechin with the active center of COX-2 is represented by hydrogen bonds with Ala199, Ala202, Thr206, and hydrophobic bonds with Tyr385, Glu203, His 388, Leu 391, Leu390, Trp 387. The interaction of epicatechin with the active center is represented by hydrogen bonds with Ala202, Tyr348, Thr206, Tyr385, Trp387, and by hydrophobic bonds with Glu203, Ala199, Leu390 (Fig. 5).

All studied compounds exhibited a high affinity for the active site of NADPH oxidase. Epicatechin (-7.11 kcal/mol) had the highest level of free energy, and (+)-catechin (-6.60 kcal/mol) was in second place. When comparing the obtained results with the epigallocatechin-3-O-gallate standard, the affinity of (+)-catechin with the active site of NADPH oxidase was 10.55% higher, and in the case of epicatechin, it was 19.00% higher than that of epigallocatechin-3-O-gallate, respectively. Moreover, we calculated the theoretical dose per kg of rat weight of the studied compounds necessary for 50% inhibition of the enzyme. Thus, the dose of (+)-catechin was 1.40 mg/kg, and for epicatechin, it was 0.59 mg/kg. The dose of epicatechin was significantly lower than that of epigallocatechin-3-O-gallate, namely 11 times lower, and in the case of (+)-catechin, it was 4.60 times less (Table 3).

The interaction of (+)-catechin with the active center of NADPH oxidase is represented by hydrogen bonds with Glu691, Ser522, Glu443, Thr462, Cys668, Phe667, and hydrophobic bonds with Pro521, Thr520, Tyr445, Pro542, Asp444, Phe 693. Epicatechin interacts with the active center of the enzyme through hydrogen bonds with Asn692, Thr462, and hydrophobic bonds with Glu691, Trp695, Thr520, Cys668, Tyr445, Pro542, Phe693. Furthermore, all tested compounds interact with flavin adenine dinucleotide (Fig. 6).

Table 3. Results of molecular docking of the compounds identified by the HPLC in the *Rubus idaeous* leaves extract with the COX-2 and NADPH oxidase structures

COX-2				NADPH oxidase			
Ligand	**ΔGbind[a] (kcal/mol)**	**Ki[b] (mmol)**	**K[c] (mg/kg)**	**Ligand**	**ΔGbind[a] (kcal/mol)**	**Ki[b] (mmol)**	**K[c] (mg/kg)**
Epicatechin	-7.20	0.00526	0.55	Epicatechin	-7.11	0.00616	0.59
(+)-Catechin	-8.40	0.00070	0.10	(+)-Catechin	-6.60	0.00	1.41
Indomethacin	-9.99	0.00005	0.006	Epigallocatechin-3-O-gallate	-5.97	0.04237	6.42

Notes: a – free-binding energy; b – inhibition constant, IC50; c – dose per kg rat weight, for 50% inhibition of the enzyme structure

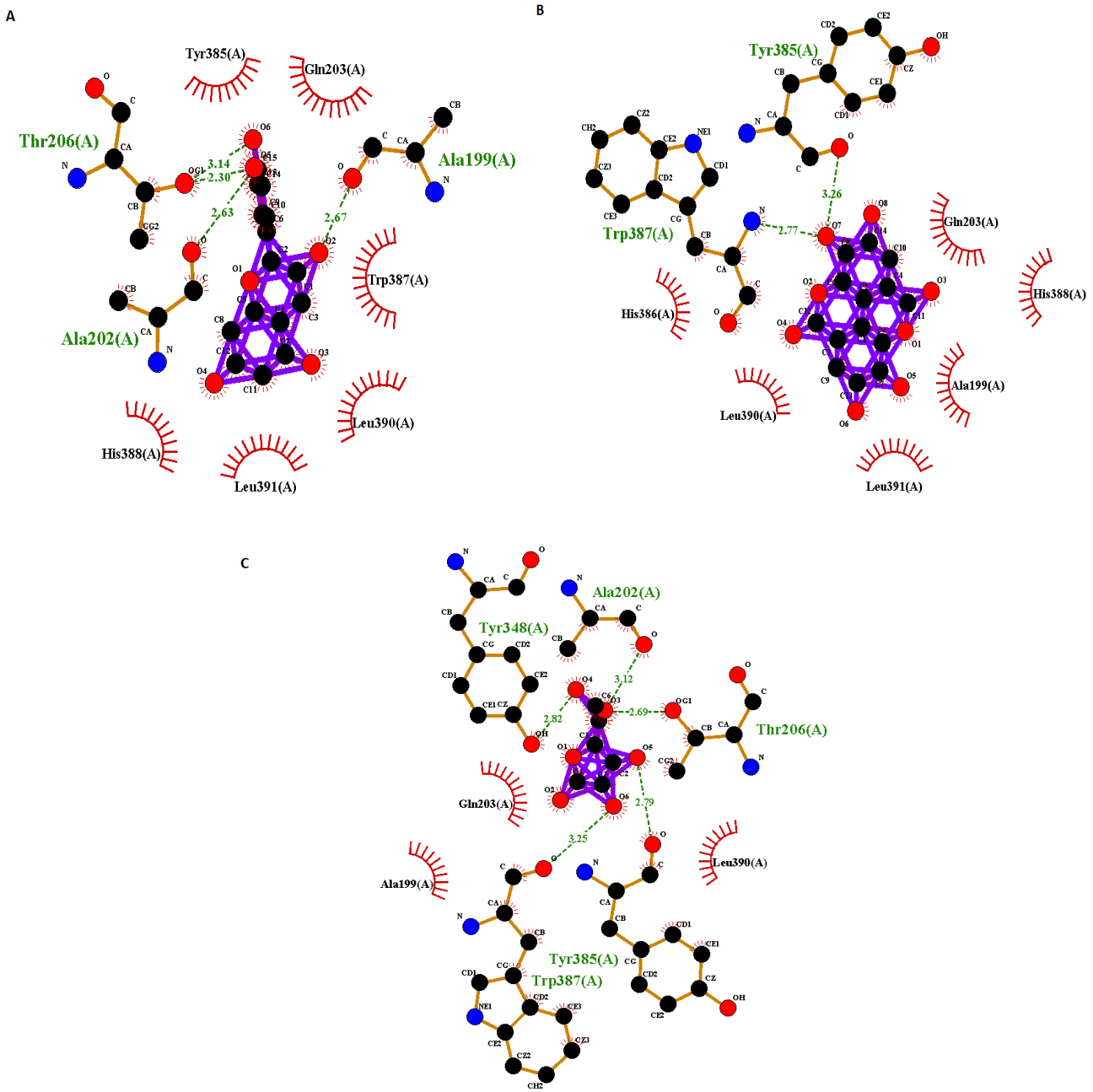

Figure 5. 2D representation of the interactions of COX-2 residues with (+)-catechin (A), ellagic acid (B) and epicatechin (C). Dashed lines—represent hydrogen bonds; red lines – hydrophobic bonds.

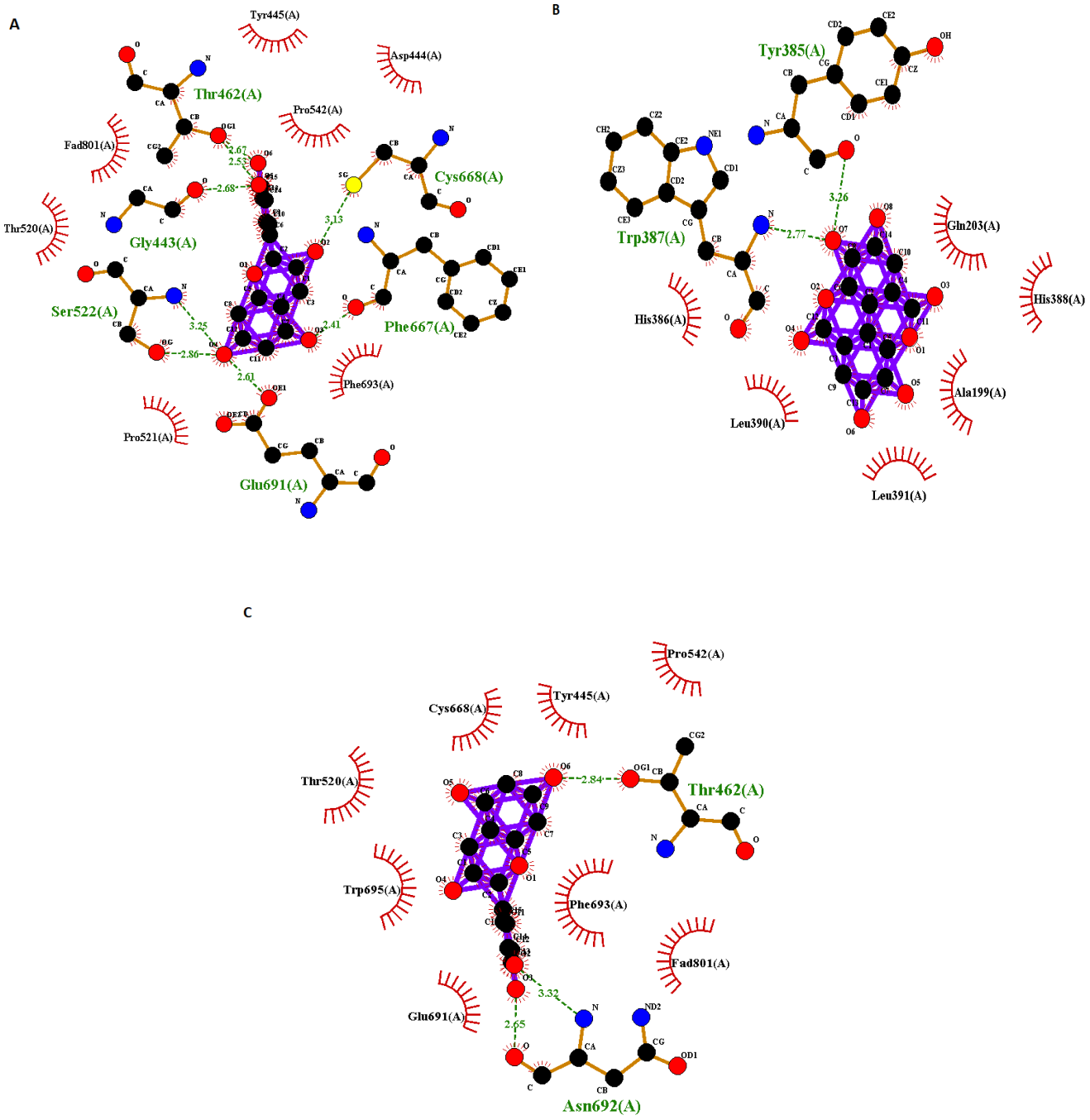

Figure 6. 2D representation of the interactions of NADPH oxidase residues with (+)-catechin (A), ellagic acid (B) and epicatechin (C). Dashed lines—represent hydrogen bonds; red lines – hydrophobic bonds.

3.3 Antioxidant activity

To compare the theoretical and practical results of the study of the antioxidant activity of the obtained extract of Rubus idaeus leaves, the antioxidant activity was studied using the potentiometric method at three levels of theoretical concentrations based on the amount of catechins in the extract: 4.00 mg/mL (double the sum of the theoretical dose of (+)-catechin and epicatechin), 2.00 mg/mL (the sum of the theoretical dose of (+)-catechin and epicatechin), and 0.20 mg/mL (half the sum of the theoretical dose of (+)-catechin and epicatechin). A 60% green tea leaf extract was used as the reference standard, as our study showed that epigallocatechin-3-O-gallate was the dominant compound. Green tea leaf extract was used in three concentrations in terms of the amount of catechins: 4.00, 2.00, and 0.20 mg/mL.

As a result of the study, it was found that at a dose of 4.00 mg/mL, the antioxidant activity of the 60% Rubus idaeus leaf extract was 1.43% higher, at a dose of 2.00 mg/mL it was higher by 1.04%, and at 0.20 mg/mL, it was 10.62% higher. According to the developed conditional classification of antioxidant activity according to Maslov [25], it was determined that the extracts at a dose of 4.00 mg/mL had an average level of antioxidant activity, whereas at doses of 2.00 and 0.20 mg/mL, the antioxidant activity level was below average (Table 4).

Table 4. Results of determination of antioxidant activity of the obtained *Rubus idaeous* leaves extracts

Sample	Concentration of catechins, mg/mL	AOA, mmol-equiv./$m_{dry\ res.}$	Conditional terms of AOA
60% raspberry extract	4.00	25.10±0.50	Middle
	2.00	12.50±0.25	Lower middle
	0.20	1.60±0.03	Lower middle
60% green tea leaves extract	4.00	24.74±0.50	Middle
	2.00	12.37±0.25	Lower medium
	0.20	1.43±0.03	Lower medium

3.4 Anti-inflammatory activity

To compare the theoretical and practical results of the study of the anti-inflammatory activity of the obtained extract of Rubus idaeus leaves, the anti-inflammatory activity was examined at three levels of theoretical concentrations, in terms of the amount of catechins in the extract: 13.0 mg/kg (twenty times the sum of the theoretical dose of (+)-catechin and epicatechin), 6.5 mg/kg (ten times the theoretical dose of (+)-catechin and epicatechin), and 0.65 mg/kg (the sum of the theoretical dose of (+)-catechin and epicatechin).

Rubus idaeus leaf extract administered at a dose of 13.0 mg/kg in rats significantly reduced paw edema by 38.8% compared to the saline group from the first hour of the test. Thereafter, edema decreased by 41.8%, 48.8%, 20.2%, and 17.8% at 2, 3, 8, and 24 hours respectively, compared with saline. Treatment with Rubus idaeus leaf extract at a dose of 6.5 mg/kg showed lower results compared to treatment at a dose of 13.0 mg/kg; in the first hour, the paw edema of mice decreased by 25.6%, and subsequently, it was possible to reduce edema by 27.2%, 36.1%, 14.1%, and 5.1% after 2, 3, 8, and 24 hours respectively, compared with saline. Treatment with Rubus idaeus leaf extract at a dose of 13.0 mg/kg showed a significant reduction in edema at 1, 2, and 3 hours post-induction compared with indomethacin, but after 8 and 24 hours, it showed less reduction in edema than indomethacin. Treatment with Rubus idaeus leaves extract at doses of 6.5 and 0.65 mg/kg was significantly less effective than treatment with indomethacin (Table 5).

Table 5. Results of determination of antioxidant activity of the obtained *Rubus idaeous* leaves extracts

Sample	Dose	% of edema inhibition compared to control during				
		1 hour	2 hours	3 hours	8 hours	24 hours
60% raspberry extract	13.0[a]	38.8±2.6	41.8±7.2	48.8±4.4	20.2±3.8	17.8±7.2
	6.5[a]	25.6±6.1	27.2±4.1	36.1±2.8	14.1±6.1	5.1±1.1
	0.65[a]	12.6±1.5	25.2±1.3	21.1±1.5	10.6±1.4	0
Indomethacin	2 mg	39.1±4.1	42.8±4.4	53.1±5.2	30.3±7.1	21.8±2.4

Note: a – mg/kg.

3.5 Correlation analysis

To confirm the hypothesis about the dependence of anti-inflammatory activity on antioxidant activity, a correlation analysis was carried out. The Pearson correlation coefficient (r) between antioxidant activity and 1, 2, 3, 8 and 24 h anti-inflammatory activity of the Rubus idaeus leaves extract was 0.9981, 0.8918, 0.9995, 0.9777 and 0.9559 (Table 6).

From the results of this correlation analysis, it is evident that there is a significant positive correlation in all cases. Therefore, the hypothesis that anti-inflammatory activity directly depends on antioxidant activity is hereby confirmed.

Table 6. Pearson`s (r) correlation coefficient between antioxidant and anti-inflammatory actions

	1 hour	2 hour	3 hour	8 hour	24 hour
r	0.9981	0.8918	0.9995	0.9777	0.9559

4. DISCUSSIONS

4.1 HPLC analysis

Ellagitannins and catechins are considered to be involved in plant defense mechanisms against threats such as insects, moths, viruses, bacteria, and herbivores. They contribute to these mechanisms by making the plant tissues unpalatable and non-nutritious, rendering them unsuitable as food sources [27]. In a recent study by Kashchenko N. et. al. [28], the aqueous extract of Rubus idaeus leaves from Siberia (Republic of Buryatia) was examined. The study found that the total polyphenols content equated to 2.60%, sanguiin H6 amounted to 0.20%, ellagic acid was 0.17%, epicatechin had a 0.05% presence, and both gallocatechin and quercetin-3-O-glucuronide each contributed 0.03% to the Rubus idaeus leaves extract. In comparison to these results, our research showed that the sum of polyphenols was 35% higher, the content of sanguiin H6 was 5% higher, and the presence of ellagic acid was 60% higher. However, the content of epicatechin was 7.4 times lower. In our extract, the content of catechin derivatives was dominant, while in the comparison extract, the content of ellagitannins and ellagic acid was found to be higher. The difference in chemical composition may be attributable to different cultivars and the vegetative phase of the plant. The growing season plays a significant role in the accumulation of bioactive substances. A study by Salminen et al. [29] examined the seasonal variation of ellagitannins and catechins in oak leaves from April to October. The study showed that the accumulation of ellagitannins exceeded that of catechins in young leaves, whereas in mature leaves, catechin content dominated. Therefore, it is possible the comparison extract was prepared from Rubus idaeus leaves collected in April or May, while our extract was prepared from Rubus idaeus leaves collected in July.

4.2 Molecular docking

One of the main links in the development of inflammation is COX-2. The primary role of COX-2 in the inflammatory process is the conversion of arachidonic acid into prostaglandins, prostocyclins, and thromboxane A2. Thus, COX-2 is an important target for studying the anti-

inflammatory activity of drugs [30].

Oxidative stress is a condition characterized by an excessive presence of reactive oxygen species, such as superoxide, hydroxyl radical, hypochlorite, and singlet oxygen. These reactive oxygen species can lead to cellular damage through oxidative stress [31]. Among the enzymes responsible for generating reactive oxygen species is NADPH oxidase, which stands out as the sole enzyme devoted exclusively to this function [32]. NADPH oxidase is composed of membrane proteins with six transmembrane domains (TM) and a cytosolic C-terminal dehydrogenase (DH) domain. The DH domain contains binding sites for flavin adenine dinucleotide (FAD) and NADPH, while the TM domains bind to two hemes. In the process of generating superoxide and other reactive oxygen molecules, the DH transfers electrons from NADPH to oxygen molecules bound to the heme moieties [33].

To obtain a theoretical dose of COX-2 and NADPH oxidase inhibition, molecular docking of (±)-catechin and epicatechin was performed at the active centers of the above enzymes.

4.3 Antioxidant activity

To assess the antioxidant activity of the obtained extracts, a potentiometric method was used. The resulting green tea leaf extract was taken as a reference standard since green tea leaves contain their gold standard - epigallocatechin-3-gallate. This compound, according to multiple studies, has a potent antioxidant effect [34].

When comparing the obtained theoretical and experimental results of antioxidant activity, it was found that in the study of antioxidant activity in silico, (+)-catechin and epicatechin showed a better result than the comparison standard epigallocatechin-3-O-gallate. Further, in the case of determining the in vitro antioxidant activity, Rubus idaeus leaves extract also had a superior result than the reference standard green tea leaf extract. These results indicate a correlation between experimental and theoretical research outcomes.

4.4 Anti-inflammatory activity

To examine anti-inflammatory activity, a carrageenan-induced mouse paw edema model was utilized. This model consists of two distinct stages: the initial stage, occurring an hour after administration, involves edema formation due to the release of vasoactive amines (histamine and serotonin) and kinins. The subsequent stage, beginning three hours after edema formation, is characterized by an increase in COX-2 activity, leading to the production of a significant number of prostaglandins and the release of NO [29].

When comparing the theoretical and experimental results of antioxidant activity, it was found that in the study of antioxidant activity in silico, (+)-catechin and epicatechin performed better than the reference standard indomethacin. When determining the antioxidant activity in vivo, Rubus idaeus leaves extract, at a dose of 13.0 mg/kg within the first two hours, exceeded the reference standard diclofenac sodium. This observation indicates a correlation between experimental and theoretical results. In our study, the Rubus idaeus leaves extract, at a dose of 13.0 mg/kg, showed better results than at doses of 6.5 and 0.65 mg/kg, since at this dose, 100% of COX-2 activity is inhibited, and at a dose of 6.5 mg/kg, only 50% is inhibited.

4.5 Correlation analysis

Srivastova et al. [35] studied the correlation between the antioxidant (ferric reducing antioxidant power assay) and anti-inflammatory activities (percent inhibition of edema in a model of ear thickness) of blackberry extracts. They reported a high positive correlation, with a Pearson's coefficient of 0.8520. In recent research by Vinodhini V. et al. [36], they investigated the phytoconstituents of Tragia Involucrata leaf extracts and evaluated their correlation with anti-inflammatory and antioxidant properties. The antioxidant activity was examined using DPPH and H2O2 assays while the anti-inflammatory analysis was carried out using a membrane stabilization assay. A strong relationship was observed between the antioxidant and anti-inflammatory

activities (r = 0.971).

Compared to these studies, our research consistently observed a very high positive correlation between antioxidant and anti-inflammatory activities (r = 0.9981 for 1 hour, r = 0.9995 for 3 hours, r = 0.9777 for 8 hours, r = 0.9559 for 24 hours). The exception was the two-hour duration of anti-inflammatory activity study where a high positive correlation was still evident but somewhat lower (r = 0.8918). Consequently, our findings affirm the hypothesis that anti-inflammatory activity directly depends on antioxidant activity.

5. CONCLUSION

The Rubus idaeus leaves extract was found to be dominated by (+)-catechin and epicatechin. It was established that (+)-catechin and epicatechin have a high level of affinity for the active sites of COX-2 and NADPH oxidase. In comparison with the antioxidant activity of green tea leaf extracts, the obtained raspberry leaf extract showed higher activity by 1.43% at a dose of 4.00 mg/mL, 1.04% at 2.00 mg/mL, and 10.62% at 0.20 mg/mL. The extract demonstrated a significant level of antioxidant and anti-inflammatory activity in both in vitro and in vivo studies. Furthermore, there was an alignment between the experimental and theoretical results in the study of antioxidant and anti-inflammatory activities. Additionally, correlation analysis confirmed the dependency of the anti-inflammatory action on the antioxidant one.

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REFERENCES

1. Biswas SK. Does the Interdependence between Oxidative Stress and Inflammation Explain the Antioxidant Paradox? *Oxidative Med Cell Longev*. 2016; 2016:1-9. Doi: https://doi.org/10.1155/2016/5698931
2. Maslov OY., Kolisnyk SV., Komissarenko NA., Kostina TA. Development and validation potentiometric method for determination of antioxidant activity of epigallocatechin-3-O-gallate. *Pharmacologyonline*. 2021; 2:35-42. Doi: https://doi.org/10.5281/zenodo.7813098
3. Maslov OY., Komisarenko MA., Golik MY., Kolisnyk SV., Altukhov AA., Baiurka SV., Karpushina SA., Tkachenko O., Iuliia K. Study of total antioxidant capacity of red raspberry (Rubus idaeous L.) shoots. *Vitae*. 2023; 30(1). Doi: https://doi.org/10.17533/udea.vitae.v30n1a351486
4. Li J., Lan T., Zhang C., Zeng C., Hou J., Yang Z., Zhang M., Liu J., Liu B. Reciprocal activation between IL-6/STAT3 and NOX4/Akt signalings promotes proliferation and survival of non-small cell lung cancer cells. *Oncotarget*. 2015; 6(2):1031-48. Doi: https://doi.org/10.18632/oncotarget.2671
5. Fan Y., Mao R., Yang J. NF-κB and STAT3 signaling pathways collaboratively link inflammation to cancer. *Protein Amp Cell*. 2013; 4(3):176-85. Doi: https://doi.org/10.1007/s13238-013-2084-3
6. Ismail WH., Abusara OH., Ikhmais B., Abul-Futouh H., Sunoqrot S., Ibrahim AI. Design, Synthesis, and Biological Activity of Coniferyl Aldehyde Derivatives as Potential Anticancer and Antioxidant Agents. *Jordan j. pharm. Sci*. 16(2):368-80. Doi: https://doi.org/10.35516/jjps.v16i2.1463

7. Yarza R., Vela S., Solas M., Ramirez MJ. c-Jun N-terminal Kinase (JNK) Signaling as a Therapeutic Target for Alzheimer's Disease. *Front Pharmacol*. 2016; 6. Doi: https://doi.org/10.3389/fphar.2015.00321
8. Gudej J. and Tomczyk M. Determination of Flavonoids, Tannins and Ellagic acid in leaves from Rubus L. species. *Arc Pharm Res*. 2004; 27(11):1114-1119. Doi: https://doi.org/10.1007/BF02975114
9. Kim M., Sutton K., Harris G. *Raspberries and Related Fruits*, second ed.; Academic Press, Oxford, UK 2016. Doi: https://doi.org/10.1016/B978-0-12-384947-2.00586-9
10. Jemal K., Sandeep BV., Pola S. Phytochemical screening and in vitro antioxidant activity analysis of leaf and callus extracts of Allophylus serratus (ROXB) KURZ. *Jordan j. pharm. sci*. 2022; 15(1):51-69. Doi: https://doi.org/10.35516/jjps.v15i1.291
11. Durgo K., Belščak-Cvitanović A., Stančić A., Franekić J., Komes D. The Bioactive Potential of Red Raspberry (*Rubus idaeus* L.) Leaves in Exhibiting Cytotoxic and Cytoprotective Activity on Human Laryngeal Carcinoma and Colon Adenocarcinoma. *J Med Food*. 2012; 15(3):258-268. Doi: https://doi.org/10.1089/jmf.2011.0087
12. Polischuk IM., Koshovyi OM., Osolodchenko TP., Komissarenko MA. The study of phenolic compounds and the antimicrobial action of the alcoholic extract from the cake of the red raspberry fruit. *Visnik Farm*. 2018; 3(95):30-3. Doi: https://doi.org/10.24959/nphj.18.2220
13. Polishchuk IM., Komisarenko MA., Golik MY., Upyr TV. The study of saponins of the raspberry cake alcoholic extract by HPLC. *Visnik Farm*. 2018; 4(96):24-7. Doi: https://doi.org/10.24959/nphj.18.2230
14. Komisarenko MA., Polischuk IM., Upyr TV., Saidov NB. Study of Amino acid composition and immunomodulatory activity of Rubus idaeus alcoholic extract. *RES J PHARM TECHNOL*. 2021; 14(3):1329-32. Doi: https://doi.org/10.5958/0974-360x.2021.00236.5
15. Ghorbanzadeh B., Mansouri M., Hemmati A., Naghizadeh B., Mard S., Rezaie A. A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. *Indian J Pharmacol*. 2015; 47(3):292. Doi: https://doi.org/10.4103/0253-7613.157127
16. Yang DJ, Liu SC, Chen YC, Hsu SH, Chang YP, Lin JT. Three Pathways Assess Anti-Inflammatory Response of Epicatechin with Lipopolysaccharide-Mediated Macrophage RAW264.7 Cells. *J Food Biochem*. 2015; 39(3):334-43. Doi: https://doi.org/10.1111/jfbc.12134
17. Maslov O., Komisarenko M., Ponomarenko S., Horopashna D., Osolodchenko T., Kolisnyk S., Derymedvid L., Shovkova Z., Akhmedov E. Investigation the influence of biologically active compounds on the antioxidant, antibacterial and anti-inflammatory activities of red raspberry (*Rubus idaeous* l.) leaf extract. *Curr Issues Pharm Med Sci*. 2022. Doi: https://doi.org/10.2478/cipms-2022-0040
18. Maslov OY., Kolisnyk SV., Komisarenko MA., Kolisnyk OV., Ponomarenko SV. Antioxidant activity of green tea leaves (*Camellia sinensis* L.) liquid extracts. *Pharmacologyonline*. 2021; (3):291-8. Doi: https://doi.org/10.5281/zenodo.7813115
19. Khoddami A., Wilkes M., Roberts T. Techniques for Analysis of Plant Phenolic Compounds. *Molecules*. 2013; 18(2):2328-75. Doi: https://doi.org/10.3390/molecules18022328
20. Morris GM., Huey R., Olson AJ. Using AutoDock for Ligand-Receptor Docking. *Curr Protoc Bioinform*. 2008; 24(1). Doi: https://doi.org/10.1002/0471250953.bi0814s24
21. RCSB PDB: Homepage. RCSB PDB: Homepage. Access: https://www.rcsb.org/.
22. PubChem. Access: https://pubchem.ncbi.nlm.nih.gov/
23. CASTp 3.0: Computed Atlas of Surface Topography of proteins. Access: http://sts.bioe.uic.edu/castp/index.html?201l
24. Maslov O., Kolisnyk S., Komisarenko M., Golik M. Study of total antioxidant activity of green tea leaves (*Camellia sinensis* L.). *Herba Pol*. 2022; 68(1):1-9. Doi: https://doi.org/10.2478/hepo-2022-0003
25. Stefanova OV. *Preclinical studies of medicinal products: method.guidance*. Kiev: Vidavnichy dim "Avitsena"; 2001:528. Doi: https://doi.org/10.5281/zenodo.8139960
26. Maslov OY., Kolisnyk SV., Komisarenko MA., Altukhov AA., Dynnyk KV., Stepanenko VI. Study and evaluation antioxidant activity of dietary supplements with green tea extract. *Curr Issues Pharm Med*. 2021; 14(2):215-9.

Doi: https://doi.org/10.14739/2409-2932.2021.2.233306
27. Salminen JP. Recent Advances in Polyphenol Research. Chichester, UK: John Wiley & Sons, Ltd; 2014. The Chemistry and Chemical Ecology of Ellagitannins in Plant-Insect Interactions: *From Underestimated Molecules to Bioactive Plant Constituents*; 83-113. Doi: https://doi.org/10.1002/9781118329634.ch4
28. Kashchenko NI., Olennikov DN., Chirikova NK. Metabolites of Siberian Raspberries: LC-MS Profile, Seasonal Variation, Antioxidant Activity and, Thermal Stability of Rubus matsumuranus Phenolome. *Plants*. 10(11):2317.
Doi: https://doi.org/10.3390/plants10112317
29. Salminen JP., Roslin T., Karonen M., Sinkkonen J., Pihlaja K., Pulkkinen P. Seasonal Variation in the Content of Hydrolyzable Tannins, Flavonoid Glycosides, and Proanthocyanidins in Oak Leaves. *J Chem Ecol*. 2004; 30(9):1693-711.
Doi: https://doi.org/10.1023/b:joec.0000042396.40756.b7
30. Lopes AJ., Vasconcelos CC., Garcia JB., Pinheiro MS., Pereira FA., Camelo DD., Morais SV., Freitas JR., Rocha CQ., Ribeiro MN., Cartágenes MD. Anti-Inflammatory and Antioxidant Activity of Pollen Extract Collected by Scaptotrigona affinis postica: in silico, in vitro, and in vivo Studies. *Antioxidants*. 2020; 9(2):103.
Doi: https://doi.org/10.3390/antiox9020103
31. Maslov OY., Kolisnyk SV., Komisarenko MA., Kostina TA., Dynnyk KV. Development the composition and technology for obtaining a dietary supplement "Cachinol" with the antioxidant activity in the form of granules used in the polycystic ovary syndrome. *News Pharm*. 2022; 103(1):42-7.
Doi: https://doi.org/10.24959/nphj.22.77
32. Drummond GR., Selemidis S., Griendling KK., Sobey CG. Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. *Nat Rev Drug Discov*. 2011; 10(6):453-71.
Doi: https://doi.org/10.1038/nrd3403
33. Chikhale R., Sinha SK., Wanjari M., Gurav NS., Ayyanar M., Prasad S., Khanal P., Dey YN., Patil RB., Gurav SS. Computational assessment of saikosaponins as adjuvant treatment for COVID-19: molecular docking, dynamics, and network pharmacology analysis. *Mol Divers*. 2021; 25(3):1889-904.
Doi: https://doi.org/10.1007/s11030-021-10183-w
34. Maslov O., Kolesnik S., Komisarenko M., Altukhov A., Dynnyk K., Kostina T. Development and Validation of a Titrimetric Method for Quantitative Determination of Free Organic Acids in Green Tea Leaves. *Pharmakeftiki*. 2021; 33(4):304–11. Doi:
https://doi.org/10.5281/zenodo.7813135
35. Srivastava A., Greenspan P., Hartle DK., Hargrove JL., Amarowicz R., Pegg RB. Antioxidant and Anti-inflammatory Activities of Polyphenolics from Southeastern U.S. Range Blackberry Cultivars. *J Agric Food Chem*. 2010; 58(10):6102-9.
Doi: https://doi.org/10.1021/jf1004836
36. Velu V., Banerjee S., Rajendran V., Gupta G., Chellappan DK., Kumar N., Fuloria S., Mehta M., Dua K., Malipeddi H. Identification of phytoconstituents of Tragia involucrata leaf extracts and evaluate their correlation with anti-inflammatory & antioxidant properties. *AntiInflammatory Amp AntiAllergy Agents Med Chem*. 2021; 20(3):308-315.
Doi:
https://doi.org/10.2174/1871523020666210126144506

تقييم النشاط المضاد للالتهابات ومضادات الأكسدة وتحليل الالتحام الجزيئي لمستخلص أوراقنبات الروبوس إيديوس

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ملخص

هدفت الدراسة إلى تحديد المركبات الأكثر وفرة في مستخلص أوراق التوت عن طريق HPLC، وإجراء دراسات نظرية وعملية لتقييم الأنشطة المضادة للأكسدة والمضادة للالتهابات في السيليكو وفي المختبر وفي الجسم الحي، والتحقيق في العلاقة بين الأنشطة المضادة للأكسدة والمضادة للالتهابات .تم تحديد كمية البوليفينول باستخدام HPLC، وتم إجراء الإرساء الجزيئي باستخدام AutoDockTools 1.5.6، وتم تحديد النشاط المضاد للأكسدة باستخدام طريقة قياس الجهد، ودراسة النشاط المضاد للالتهابات باستخدام طريقة الوذمة الكاراجينية .وُجد أن المستخلص غني بمادة الإبيكاتشين (0.417%)و-(+)كاتشين (0.501%)والإيلاجوتانين .(0.401%)كانت الطاقة الحرة لـ - (+)كاتيشين وإبيكاتشين -8.40و -7.20على التوالي للمواقع النشطة لأنزيمات الأكسدة الحلقية(COX-2) 2-، و -6.60و -7.11لأنزيمات أوكسيديز فوسفات نيكوتيناميد الأدينين أدينين ثنائي النوكليوتيد .(NADPH oxidase)كان النشاط المضاد للأكسدة لمستخلص أوراق التوت أعلى بنسبة 1.43%و 1.04%و 10.62%من مستخلص أوراق الشاي الأخضر لجرعات 4.00 و 2.00و 0.20ملغم/ملل على التوالي .أظهر العلاج بمستخلص أوراق التوت بجرعة 13.0ملغم/كغم انخفاضًا ملحوظًا في الوذمة بعد 1و 2و 3ساعات بنسبة 38.8%و 41.8%و 48.8%على التوالي مقارنة بالمجموعة الضابطة .كشفت الدراسة عن وجود تطابق بين النتائج التجريبية والنظرية في تقييم الأنشطة المضادة للأكسدة والمضادة للالتهابات .أكد تحليل الارتباط اعتماد النشاط المضاد للالتهابات على النشاط المضاد للأكسدة.

الكلمات الدالة: روبوس ايديوس ل.، ورقة، HPLC، الالتحام الجزيئي، النشاط المضاد للأكسدة، النشاط المضاد للالتهابات، الارتباط.

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Comparative Analysis of Histamine in Fresh and Processed Fish Sold in Jordanian Market

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ABSTRACT

Food poisoning from histamine, a biogenic amine formed due to the decarboxylation of histidine by bacteria in fish and fish products, has become a pivotal concern in food safety. This study measured the concentration of histamine in various fish products available in the Jordanian market, but manufactured in multiple countries, utilizing an Enzyme-Linked Immunosorbent Assay (ELISA). The ELISA kit and the protocol were provided by Veratox for histamine. Approximately 93.69% of the samples tested positive for the presence of histamine, with levels ranging between 0.317 and 230.41 mg/kg. Solely 0.9% of the samples exceeded the maximum permissible level established by the European Union (EU) and only 4.5% of the fish samples were free of histamine. The Principal Component Analysis (PCA) revealed that the type of fish was the most significant source of variability in histamine concentration, explaining 31.2% of the variability. Conversely, the sample weight accounted for the least variability (only 20.2%), implying that it has little or no effect on the concentration of histamine in the fish samples.

Keywords: ELISA, fish samples, food safety, histamine.

INTRODUCTION

Fish are a rich source of essential minerals and proteins, which can meet human nutritional requirements, promoting healthier living (1)(2). It is found that the protein in fish has higher nutritional value than milk, meat, or eggs (3). Additionally, fish provides a good source of omega-3, calcium, phosphorus, iron, and trace minerals. However, the inherent quality protein and polyunsaturated fatty acids in fish make them highly perishable, leading to a reduced shelf life (4). The presence of protein and free amino acids in fish may further decrease freshness due to various enzymatic, biochemical, and microbial activities (5).

Biogenic amines such as histamine, beta-phenylethylamine, tyramine, tryptamine, putrescine, cadaverine, spermine, and spermidine are commonly found in foods and beverages (6). Histamine, in particular, is a biogenic amine produced in fish as a result of the decarboxylation of histidine amino acids by certain bacteria, notably Morganella morganii and Photobacterium phosphoreum (7)(8). This substance can also form through the decarboxylation of amino acids or the amination and transamination of aldehydes and ketones (9).

Exposing fish to elevated temperatures during harvesting and transportation can promote the growth of histidine decarboxylase-producing bacteria, consequently increasing histamine concentration (10). The metabolic activities of

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bacteria convert histidine to histamine in fish, which accumulates in the fish flesh due to a lack of a homeostasis system. To counter this problem, the fish should be iced immediately after they are out of the water (11).

Histamine fish poisoning is often found in species like mackerel, tuna, sardines, sockeye salmon, and amberjack (12). However, the histamine content may change based on factors such as feeding season, fish species, the stage of maturity, and sex (13).

The food-borne chemical intoxication caused by ingesting fish containing high histamine concentrations is termed Histamine Food Poisoning (HFP). This condition can lead to food intoxication and intolerance, and provoke allergic reactions, such as headaches, nausea, acute anaphylaxis, generalized erythema, dyspnea, vomiting, and other discernible symptoms (11, 14, 15). Health effects of histamine may further include mutagenic and carcinogenic actions (16), and it has been observed that normal metabolic activities do not detoxify histamine intoxication (17). The European Commission Regulation (EU) No 1019/2013 on histamine in fishery products (18) stipulates specific criteria for histamine presence. It sets a maximum level of 200 mg/kg and 400 mg/kg of histamine respectively in fresh fish and fish products that have undergone enzymatic maturation or been treated in brine (5, 13). An accurate, fast, and reproducible analytical method with high throughput is required to ascertain the concentration level of histamine in fish and fish products for regulatory compliance and quality control.

The primary analytical methods for determining histamine in fish and fish products include enzyme-linked immunosorbent assay (ELISA) (19), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) (20), capillary electrophoresis (CE) (4, 5, 21), and gas chromatography-tandem mass spectrometry (GC/MS) (7). Among these, the ELISA method is most commonly utilized for histamine detection because chromatographic methods involve laborious and time-consuming steps for sample pretreatment such as extraction and concentration, along with the necessity of derivatization in gas chromatography (7, 22). This study aims to determine histamine concentration levels in fish samples using ELISA and provide knowledge about the levels of histamine in marketed processed fish in Jordan. This information will improve the understanding of product safety among Jordanians and the health risks associated with the consumption of canned fish.

MATERIALS AND METHODS

Sample Collection

A total of 16 different types of fish samples were collected from 15 different countries, including Belgium, China, Egypt, Indonesia, Italy, Jordan, Morocco, Norway, the Philippines, Thailand, the United Arab Emirates (UAE), and the United States of America (USA). These consisted of nine salmon samples, eight sardines in hot oil samples, seven sardines in hot oil samples, one sardine in olive oil sample, one sardine in vegetable oil sample, one tuna chunk sample, three tuna chunks in hot oil samples, 18 tuna chunks in oil, one tuna chunk in olive oil sample, two tuna chunks in water samples, 16 tuna in hot oil samples, three tuna in olive oil samples, 31 tuna in vegetable oil samples, six tuna in water samples, three tuna with spices samples, and one white tuna in vegetable oil sample. This resulted in an aggregate of 111 samples. Prior to analysis, these samples were stored at temperatures between 11-18°C in a freezer.

Samples preparation and extraction

The sample preparation and extraction process comprised several steps. Firstly, the entire contents of each can, including meat and liquid, were transferred into a blender and homogenized. The blended samples were then stored at a temperature of 2-8°C. Roughly 10 grams of the homogenized sample were weighed into a 125 mL disposable extraction bottle, containing 90 mL of distilled water. This bottle was capped tightly and vigorously shaken for 20 seconds to resuspend the fish tissue completely. It was shaken again for 20 seconds after a 5-

minute interval, and the tissue was then left to settle at the bottom of the bottle. The mixture was centrifuged, and the supernatant was transferred into a clean test tube. Lastly, about 100 µL of the supernatant, or extract, was pipetted into a test tube containing 100 mL of diluent buffer, and the mixture was gently swirled.

ELISA test

All reagents were allowed to reach a temperature range of 18-30°C before use, and the ELISA test was conducted per the manufacturer's instructions.

A red-marked mixing well was set aside for each sample to be tested along with five red-marked wells for controls, and these were placed in the well holder. An equivalent number of antibody-coated wells were also extracted. Unused antibody wells were returned to the foil pack with the desiccant and resealed. All reagents were mixed by swirling the respective bottles prior to use.

The conjugate (100µL) from the blue-labeled bottle was added to each red-marked mixing well. A fresh pipette tip was used to introduce 100µL of the controls and diluted samples into the red-marked mixing wells. Utilizing a 12-channel pipettor, the liquids were mixed in the wells by pipetting up and down three times. 100µL was then transferred to the antibody-coated wells.

The antibody-coated wells were incubated for 10 minutes at room temperature, 18-30°C (64-86°F). This was followed by stirring for the initial 10-20 seconds by sliding the microwell holder back and forth on a flat surface without splashing. The red-marked mixing wells were then discarded. The content of the antibody wells was poured out, each well was filled with diluted wash buffer and emptied three times. After turning the wells upside down, any remaining liquid was blotted onto an absorbent towel.

The required volume of substrate was transferred from the green-labeled bottle into the green-labeled reagent boat. A 12-channel pipettor with new tips was used to pipette 100µL of the substrate into the wells. These were incubated for 10 minutes at room temperature, 18-30°C (64-86°F), and stirred for the first 10-20 seconds. Any remaining substrate was discarded and the reagent boat was filled with water. The same volume of the Red Stop solution from the red-labeled bottle was added to the red-labeled reagent boat. Using the same pipette tips as for substrate dispensation, 100µL of Red Stop was introduced into each well and mixed.

After wiping the bottom of the microwells with a dry cloth or towel, the microwells were read using a 650 nm filter in a microwell reader. Data was analyzed using the Neogen Veratox software, which compared the results to the standard curve to calculate the final readings. It's crucial to ensure no air bubbles form, as this might affect the results. These results should be read within 20 minutes of test completion. Finally, all used materials should be safely disposed of.

Statistical analysis

The concentration of histamine in fish samples was evaluated in triplicate and randomly analyzed. Principal Component Analysis (PCA) was used to determine the relationships (23) between the histamine concentration levels in fish samples, their country of origin, trademark, and sample weight.

Results and Discussion

The concentration of histamine in both imported and locally canned and processed tuna fish products available in Jordanian markets was successfully assessed. Histamine was detected in approximately 93.69% of the samples, ranging between 0.317 and 230.41 mg/kg. In contrast, histamine was not detected in 6.31% (7 samples) of the samples. The histamine-free fish samples primarily originated from Thailand, consisting of five samples (two Sardines in Oil, one Tuna Chunk, one Tuna Chunk in Oil, and one Tuna Chunk in Hot Oil). The remaining two histamine-free samples were Tuna Chunks in Oil from Egypt and Tuna Chunks in Vegetable Oil from Oman. The highest level of histamine, detected at a concentration of 230.41 mg/kg, and the lowest level, found at a concentration of 0.32 mg/kg, were both in 'Tuna in Vegetable Oil' samples from Thailand. Only one sample (0.9%) out of the 111 surpassed the permissible histamine

level of 100 mg/kg.

The highest average histamine concentration of 35.14 mg/kg was found in 'Sardines in Vegetable Oil' (refer to Table 1), whereas the lowest average concentration of 0.72 mg/kg was detected in 'Tuna Chunks in Olive Oil' samples.

Table 1: Average concentration of histamine in fish samples

Fish	Number of samples	Concentration (±SD mg/kg)	Lowest level (mg/kg	Highest level (mg/kg)
Salmon	9	10.34±21.66	0.063	65.15
Sardines in hot oils	8	13.01±15	n.d	44.29
Sardines in oil	7	10.28±12.53	n.d	33.62
Sardines in olive oil	1	7.06±0.00	-	-
Sardines in vegetables	1	35.14±0.00	-	-
Tuna chunks	1	0.00	-	-
Tuna chunks in hot oil	3	0.81±1.30	0.004	2.31
Tuna chunks in oil	18	10.61±12.57	n.d	35.06
Tuna chunks in olive oil	1	0.72±0.00	-	-
Tuna chunks in water	2	3.58±4.43	0.45	6.70
Tuna in hot oil	16	18.02±22.26	n.d	70.14
Tuna in olive oil	3	11.12±10.41	0.47	21.26
Tuna in vegetable oil	31	17.98±41.82	n.d	230.41
Tuna in water	6	22.45±30.42	0.21	77.89
Tuna with spices	3	6.42±5.76	1.57	12.79
White tuna in vegetable oil	1	12.19±0.00	-	-

The results obtained in this study were higher than those reported by Bangieva and colleagues, who recorded a concentration of 13.51 mg/kg in fresh and marine water fish sourced from Bulgarian markets, analyzed using the ELISA method (13). The results were also higher than the 0.211 mg/kg reported by Yusni and colleagues in tuna fish obtained from the Indonesian fishing port (24). However, our levels were lower than the 480.25 mg/kg reported by Diniz and colleagues in commercial seafood collected from Portuguese markets (25).

Our results are comparable to those found by Learoussy and colleagues, who detected histamine levels ranging from 2.74 to 156.60 mg/kg in commonly consumed frozen fish in Mauritania, collected between January and June 2019 (26). Our study agrees with the concentration of histamine ranging from 1.3 to 290 mg/100g found in freshwater fish from Bahir Dar markets in Ethiopia (27). The range is also compatible with the concentration of 17 to 210 mg/100g detected in canned tuna fish sourced from Isfahan, Iran (15).

Table 2: Eigenanalysis of the correlation matrix

Eigenvalue	1.2484	1.0563	0.8866	0.8087
Proportion	0.312	0.264	0.222	0.202
Cumulative	0.312	0.576	0.798	1.00
Variable	PC1	PC2	PC3	PC4
Fish type	0.581	0.379	-0.303	-0.653
Trademark	-0.540	0.402	0.545	0.500
Origin	0.325	-0.706	0.517	-0.357
Weight	-0.513	0.444	0.587	0.442

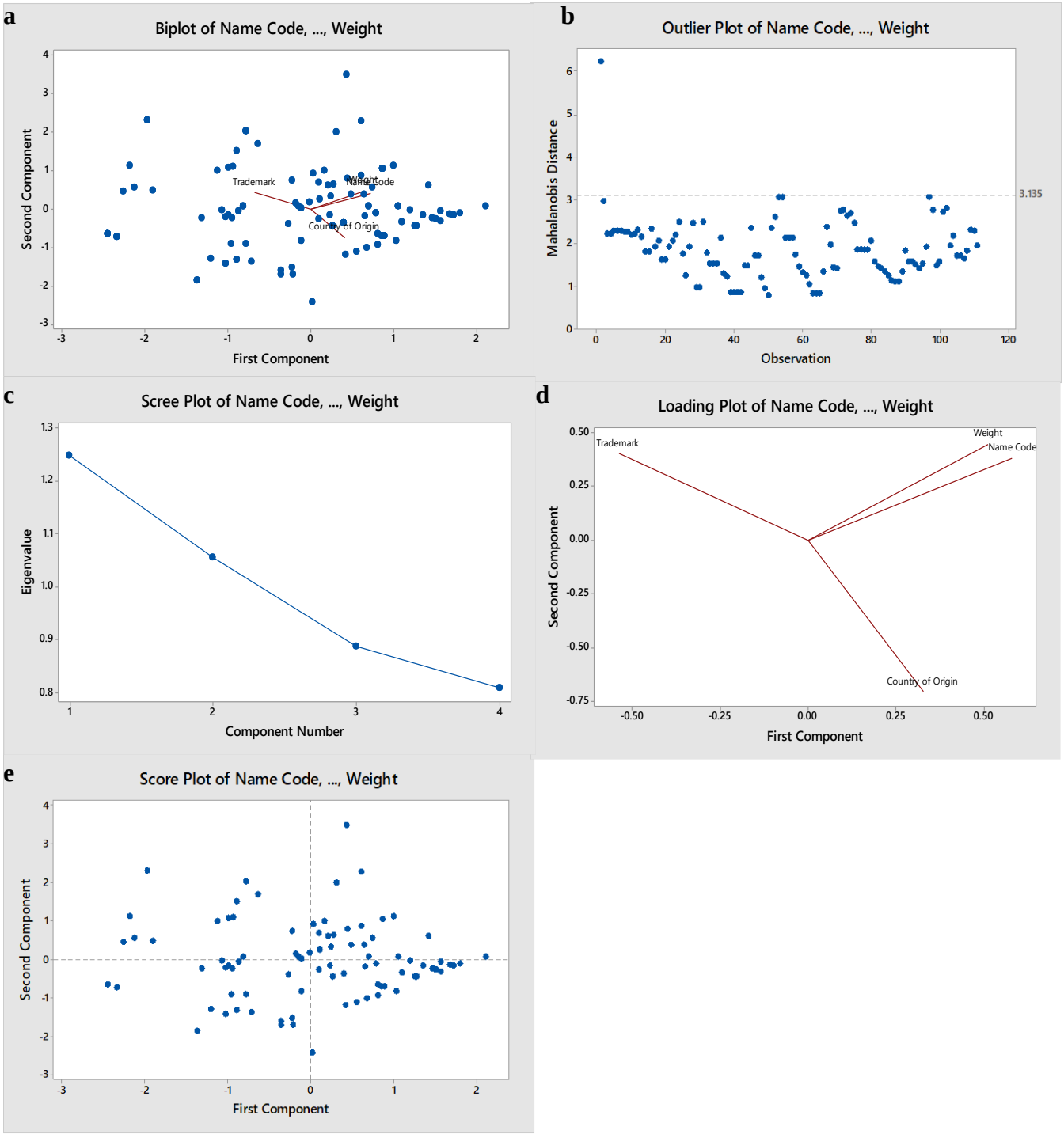

Figure 1: Principal components analysis (PCA) (a) biplot, (b) outlier plot (c) scree plot (d) loading plot and (e) score plot of histamine in fish samples

The Principal Component Analysis, illustrated in Figures a-e and in the Eigen analysis of the correlation matrix (Table 1), showed that the first two principal components have more than 57% impact, with eigenvalues greater than 1 and an accumulative value of 0.567, i.e., 56.70%. The type of fish posed the greatest source of variability and accounted for 31.2% of the variability in the data, making it a crucial component. The least variability was observed in the weight of the samples, displaying a 20.2% contribution to the data variability.

The type of fish had a larger positive association with the weight of the samples, while the trademark had a significant positive association with the country of origin. Conversely, the type of fish demonstrated a larger negative relationship with the trademark. The trademark also exhibited a larger negative association with the country of origin. Meanwhile, the sample weight showed larger negative interactions with the type of fish, the trademark, and the country of manufacture.

CONCLUSION

The histamine concentration identified in various fish was found to be lower than the level deemed safe and tolerable, posing no health risk to consumers, with the exception of one sample that surpassed the permissible limit. The formation of histamine in fish can be curtailed through proper preservation measures, prompt cooling, and efficient refrigeration during processing. These measures can either reduce or completely inhibit microbial activities that can potentially lead to histamine formation. The application of the ELISA kit Veratox for Histamine has proven to be a rapid and accurate means for determining histamine levels in a broad variety of fish species.

Additionally, the use of Principal Component Analysis (PCA) was beneficial in assessing the relationship between histamine concentration and variables such as types of fish, the manufacturer's country of origin, and the weight of different fish products. Future research could explore the use of artificial neural networks (ANN) and partial least squares regression (PLSR) to determine the various environmental factors influencing histamine formation in fish.

REFERENCES

1. Ajayi OT., Ajasin FO., Ajayi YP., Sonibare FO., Ojezele M., Oliyide AO., et al. Comparative analysis of histamine levels in commonly consumed frozen fish types in southwest Nigeria. *Science Innovation*. 2014; 2(3):22-6.
2. Adeyeye EI. Determination of the chemical compoisition of the nutritionally valuable parts of male and female common west Africa freshwater crabs *Sudanaraules africanus*. *International Journal of Food, Science and Nutrition*. 2002; 53:189-96.
3. Khashroum AO. Impact of climate change and dry-salting preservation on histamine levels in three marine fish species. *Jordan Journal of Agricultural Sciences*. 2022; 18(1):51-8.
4. Amani H., Karmani MH., Safari O., Vakilchap F., Atash MMS. A comparative study on histamine level of refrigerated trout fillets using competitive ELISA and HPLC methods. *Journal of Food Chemistry & Nanotechnology*. 2018; 4(2):33-7.
5. Muscarella M., Magro SL., Campaniello M., Armentano A., Stacchini P. Survey of histamine levels in fresh fish and fish products collected in Puglia (Italy) by ELISA and HPLC with fluorimetric detection. *Food Control*. 2013; 31:213-7.
6. Doyle MP., Beuchat LR., Monttville TJ. Food microbiology: fundamentals abd frontiers. *Washington DC: ASM Press*; 1997; 872.
7. Li Y-F., lin Z-Z., Hong C-Y., Huang Z-Y. Histamine detection in fish samples based on indirect competitive ELISA method using iron-cobalt co-doped carbon dots labeled histamine antibody. *Food Chemistry*. 2021; 345.
8. Masashi K., Tomoko Y., Masanori I., Teizo T. Photobacterium phosphoreum caused a histamine fish poisoning incident. *International Journal of Food Microbiology*. 2006; 92:79-87.
9. Pawul-Gruba M. and Osek J. Identification of histamine in fish and fish products in Poland during 2014–2018. *Journal of Vetrenary Research*. 2021; 65(4):483-6.

10. Economou V., Brett M., Papadopoulou C., Frillingos S., Nichols T. Changes in histamine and microbiological analyses in fresh and frozen tuna muscle during temperature abuse. *Food Additives & Contaminants*. 2007; 24(8):820-32.
11. Sadeghi N., Behzad M., Jannat B., Ovelsi M., Hajimahmoodi M., Mozafazri M. Determination of histamine in canned tuna fish available in Tehran market by ELISA method. *Journal of Food Safety and Hygiene*. 2019; 5(1):46-50.
12. Ayesh AM., Murad HA., Sultan YY., Hegazy AM., Bazaraa WA. Evalutaion of some methods of histamine determination to be used in HACCP system implementation of sardine canning process. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2014; 5(6):1574-86.
13. Bangieva D., Stratev D., Stoyanchev T. Histamine level in freshwater and marine fish sold in Bulgarian markets. *Journal of Food Qualaity & Hazards control*. 2020; 7:196-9.
14. Emborg J. and Dalgaard P. Formation of histamine and biogenic amines in cold-smoked TTuna: an investigation of psychrotolerant bacteria from samples implicated in cases of histamine fish poisining. *Journal of Food Protection*. 2006; 69:897-906.
15. Rahimi E., Nayebpour F., Alian F. Determination of histamine in canned tuna fish using ELISA. *American-Eurasian Journal of Toxicological Sciences*. 2012; 4(2):64-6.
16. Önal A. A review: Current analytical methods for the determination of biogenic amines in foods. *Food Chemistry*. 2007; 103(4):1475-86.
17. Sallam KI., Morgan SEM., Sayed-Ahmed MZ., Alqahtani SS., Abd-Elghany SM. Health hazard from exposure to histamine produced in ready-to-eat shawarma widely consumed in Egypt. *Journal of Food Composition and Analysis*. 2021; 97:103794.
18. EU. Commission Regulation (EU) No 1019/2013 of 23 October 2013 amending Annex I to Regulation (EC) No 2073/2005 as regards histamine in fishery products (Text with EEA relevance). *Official Journal of the European Union*. 2019; 282:46-7.
19. Melhem SJ., Aiedeh KMA., Hadidi KA., FerasAlali. The Determination of 25-OHVitamin (D2/D3) in Human Serum by Liquid Chromatography Tandem Mass Spectrometry with Comparison to IDS Enzyme Immunoassay. *Jordan j. pharm. sci*. 2013; 6(2).
20. Sirhan AY., AlRashdan Y., Abbasi NU., Mostafa A., Abudayeh Z., Talhouni A., et al. Optimization and Validation of HPLC-UV Method for the Determination of Vardenafil, Sildenafil, and Tadalafil in Honey-Mixed Herbal Sachets Using a Design of Experiment. *Jordan j. pharm. sci*. 2023; 16(1):148-62.
21. Muscarella M., Iammarino M., Centonze D., Palermo C. Measurement of Histamine in Seafood by HPLC, CE, and ELISA: Comparison of Three Techniques. *Veterinary Research Communications*. 2005; 29(2):343-6.
22. Munir MA. and Badri KH. The importance of derivatizing reagent in chromatography applications for biogenic amine detection in food and beverages. *Journal of Analytical Methods in Chemistry*. 2020; 2020:1-14.
23. Filipec SV., Valinger D., Mikac L., Ivanda M., Kljusurić JG., Jančі T. Influence of Sample Matrix on Determination of Histamine in Fish by Surface Enhanced Raman Spectroscopy Coupled with Chemometric Modelling. *Foods* (Basel, Switzerland). 2021; 10(8):1767.
24. Yusni E., Zai KES., Zulkifli Z. Analysis of histamin content in tuna fish *Thunnus* sp. with Elisa method at fishing port of Belawan, North Sumatra, Indonesia. *IOP Conference Series: Earth and Environmental Science*. 2019; 348.
25. Diniz MS, Madeira C, Noronha JP. Survey of biogenic amines (histamine and spermidine) in commercial seafood by enzyme linked immunosorbent assay (ELISA). *Annals of Medicine*. 2021; 53(sup1):S19-S.
26. Learoussy HY, Tfeil H, Dartige AY, Coulibaly SM, Aarab L. Determination of histamine level in commonly consumed frozen fish in Mauritania. *Food Research*. 2023; 7(1):292-6.
27. Berjia FL, Brimer L. Determination of Histamine in Freshwater Fish Using ELISA Method: A Food Safety Concern. *American-Eurasian Journal of Toxicological Sciences*. 2013; 5:94-6.

التحليل المقارن للهستامين في الأسماك الطازجة والمعالجة

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ملخص

أصبح التسمم الغذائي بالهستامين، وهو حمض أميني حيوي يتشكل نتيجة نزع كربوكسيل الهيستيدين بواسطة بكتيريا نزع الكربوكسيل في الأسماك والمنتجات السمكية، مشكلة أمنية في مجال سلامة الأغذية. في هذه الدراسة، تم تحديد تركيز الهستامين في منتجات الأسماك المختلفة التي تم الحصول عليها في السوق الأردني ولكن تم تصنيعها في بلدان مختلفة باستخدام مقايسة الامتصاص المناعي المرتبط بالإنزيم (ELISA)، باستخدام مجموعة ELISA وبروتوكول مقدم من Veratox للهستامين. تم الكشف عن الهستامين في حوالي 93.69% من العينات، بحيث تراوحت الكمية بين 0.317 و230.41 ملغم/كغم، مع تجاوز 0.9% فقط من عينات الأسماك الحد الأقصى المسموح به الذي حدده الاتحاد الأوروبي و4.5% فقط من هذه العينات كانت خالية من الهستامين. أظهر تحليل المكونات الرئيسية (PCA) أن المتغير الخاص بنوع الأسماك أدى الى أعلى مصدر للتباين (31.2%)، وهو عامل مهم في تحديد مستوى تركيز الهستامين. ولوحظ أيضا تباين أقل في المتغير الخاص بوزن العينات (20.2%) وبالتالي كان له تأثير ضئيل أو معدوم على تركيز الهستامين في عينات الأسماك.

الكلمات الدالة: مقايسة الامتصاص المناعي المرتبطة بالإنزيم، عينات الأسماك، سلامة الغذاء، الهستامين.

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Patterns of Antibiotic Use, Knowledge, and Perceptions among Jordanian Population: A Cross-sectional Study

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ABSTRACT

Background: Practices concerning antibiotic use have detrimental impacts on body immunity, bacterial resistance, and the overall health of the general population. Misconceptions regarding antibiotic use can lead to improper use and malpractice, posing numerous health threats.

Objectives: This study aims to assess the knowledge, practices, and awareness of Jordanian adults about antibiotics. This includes an analysis of their understanding of antibiotic uses and the sources from which they acquire such knowledge. Methods: A representative sample of Jordanian adults was invited to participate in an online survey distributed through social media platforms.

Key findings: The results showed that 77.6% of the participants had received an antibiotic at least once in the past 12 months. About two thirds reported no problems with using a different brand name (alternative) for the prescribed antibiotic. The majority of respondents trust the decisions made by pharmacists. Moreover, participants with higher ages, female participants, those with a higher level of education, uninsured participants, and those with a medicine-related degree showed better antibiotic knowledge compared to others ($P<0.05$ for all).

Conclusion: There is a good level of antibiotic-related knowledge amongst Jordanian adults. However, prevalent misconceptions and improper use also exist, indicating the need for focused attention on correcting such practices that might adversely impact the health of the community as a whole.

Keywords: Awareness; perception; antibiotics; Use; Jordan.

INTRODUCTION

Antibiotics are drugs that fight infections of bacterial origin, as defined by the Food and Drug Administration (FDA) (1). They are arguably among the most successful forms of chemotherapy in the history of medicine (2) and have been found to be among the most commonly sold classes of drugs from community pharmacies in developing countries (3). Despite antibiotics being classified as prescription-only medicines, they are often dispensed to patients without a medical prescription in various parts of the world (4). In particular, in various Middle Eastern countries, antibiotics can be easily obtained over the counter, potentially contributing to the high prevalence of self-treatment with antibiotics (2). According to legislations issued by the Jordanian Food and Drug Administration (JFDA), antibiotics should be strictly prescribed by doctors. Unfortunately, these regulations are not fully enforced (5,6). Consequently, this irrational dispensing of antibiotics could result in an increase in bacterial resistance, posing global health threats.

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Even with the development of new antimicrobial agents against these resistant strains, bacteria will likely acquire resistance to these modified versions over time through the horizontal acquisition of novel resistance mechanisms (2). In Jordan, given the importance of this topic, researchers have extensively explored the area of antibiotic-related knowledge and perceptions, sometimes targeting the public and other times healthcare providers (7,8,9,10,11). A 2018 cross-sectional study evaluated the general public's knowledge of antibiotic use and bacterial resistance (10). Another study assessed parents' views on antibiotic use in their children (11). Regarding healthcare providers, some studies targeted pharmacists' knowledge and behavior regarding antibiotics (7,9), as well as the attitudes of dental practitioners (8). All of these studies found unsatisfactory levels of knowledge and perception regarding proper antibiotic use and a significant rate of self-medication with antibiotics among different study groups (7,8,9,10,11).

While numerous studies have examined antibiotic-related knowledge and perceptions over time in Jordan, this area is expected to evolve as perceptions change after numerous governmental efforts to increase awareness about this global issue. Accordingly, these changes necessitate continuous tracking, especially given the recent increase in advising and instructing patients on appropriate antibiotic use in healthcare facilities and among providers in Jordan. Thus, an in-depth, recent evaluation of the general Jordanian community was needed to assess these aspects. Our research aims to evaluate the level of knowledge and perceptions of the Jordanian population towards antibiotics, as well as their pertinent practices.

METHODS

Study design, settings, and participants

This is a descriptive, cross-sectional, survey-based study conducted between May and June 2022, to assess the public's knowledge, perceptions, and practices towards antibiotics. In this study, a convenience sample of Jordanian individuals was invited to participate by sending a survey link through social media platforms (Facebook and WhatsApp). The inclusion criteria were adult subjects (≥ 18 years) who are residing in Jordan. Participants were informed about the aim of the study, and they were asked to provide a written consent form before completing the survey, which takes approximately ten minutes.

Sample size calculation

The sample size was calculated based on the number of subjects per predictor needed to conduct a linear regression analysis, as recommended by Tabachnick and Fidell (5-20 subjects per predictor) (12). Using 20 subjects per predictor and assuming we have six predictors, a minimum sample size of 120 was considered representative. We aimed to recruit a larger number of subjects to enhance the power of the study.

Survey development and validation

The draft questionnaire was created by study researchers to assess the public knowledge, perceptions, and practices of the Jordanian population regarding antibiotics. Then, face and content validity for the draft questionnaire were conducted by a group of experts in clinical pharmacy. These experts' comments were compiled and evaluated, leading to minor modifications in the draft questionnaire. These changes were not included in the final analysis. The finalized questionnaire consisted of five sections: the first section collected participants' demographics. The second section assessed participants' previous experiences with antibiotics. The third section contained multiple-choice questions about their practices with antibiotics. The fourth section included questions about participants' knowledge of antibiotics, and their sources of antibiotic-related information, if any. The last section aimed to understand participants' general perceptions of antibiotic usage using five-point Likert scale questions. The survey was translated and distributed in Arabic, the native language of the Jordanian people, using a forward translation and back translation approach. For the knowledge section, participants' knowledge was assessed with 12

true/false statements. For each statement, participants received one point for a correct answer, and no points for an incorrect answer. The total knowledge score of each participant was then calculated out of 12.

Ethical consideration

The Ethics Committee provided Institutional Review Board (IRB) approval at Applied Science Private University (Approval number: 2022-PHA-11). This study adhered to the World Medical Association Declaration of Helsinki guidelines (13). Participants were informed that their responses would remain anonymous, their data would be kept confidential, and that their participation was entirely voluntary.

Statistical analyses

Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) version 22 (SPSS Inc., Chicago, IL, USA). Median/interquartile range (IQR) and frequency/percentage were employed for the descriptive analysis of continuous and qualitative variables, respectively. Independent factors that may affect participants' knowledge about antibiotics were investigated using linear regression analysis. Following simple linear regression, any variable with a P-value ≤ 0.25 was considered eligible for entry into multiple linear regression analysis. All variables were checked for an absence of multicollinearity before conducting the multiple linear regression analysis (i.e., Pearson correlation coefficient <0.9 for any two variables). A P-value of ≤0.05 was deemed statistically significant when identifying factors affecting participants' knowledge about antibiotics.

Results

During the study period, 533 individuals were approached and invited to participate in the study. The participants had a median age of 33 years (IQR= 23.0). More than two-thirds of the survey participants were females (n= 369, 69.2%). Also, 67.4% held a university degree (n= 359). About half of the participants were married (n= 244, 45.8%), and over 62% were medically insured (n= 322, 62.3%). In addition, 34.7% held a medicine-related degree (n= 185). For more details about participants' sociodemographic characteristics, please refer to Table 1.

Table 1. Sociodemographic characteristics of the study participants (n= 533)

Parameters	Median (IQR)	n (%)
Age (years)	33.0 (23.0)	
Gender		
• Male		164 (30.8)
• Female		369 (69.2)
Educational level		
• Not educated		39 (7.3)
• School level		63 (11.8)
• University graduate		359 (67.4)
• Post-graduate		72 (13.5)
Marital status		
• Married		244 (45.8)
• Others (Single, divorced, or widowed)		289 (54.3)
Medical insurance		
• No		201 (37.7)
• Yes		332 (62.3)
Do you have a medical-related degree?		
• No		348 (65.3)
• Yes		185 (34.7)

IQR: Interquartile range

Table 2 summarizes participants' previous history of using antibiotics. The majority of respondents reported taking antibiotics during their lifetime (n=512, 96.1%), with roughly 77.7% of them (n=414) having received antibiotics at least once during the past 12 months. Additionally, about 80% of the participants stated that some of their family members had received antibiotics during the past 12 months. In fact, approximately three-quarters of the participants revealed that they obtain their antibiotics with prescriptions (n=393, 73.7%), but only 60.0% of them (n=320) reported that they always complete the course of antibiotics.

Table 2. Participants previous history of using antibiotics (n= 533)

Parameters	n (%)
Have you ever taken antibiotics?	
• Yes	512 (96.1)
• No	11 (2.1)
• I don't know.	10 (1.9)
How many times have you received antibiotics during the past 12 months?	
• Never	119 (22.3)
• once	191 (35.8)
• 2-5 times	166 (31.1)
• More than 5 times	57 (10.7)
Have any of your adult family members received antibiotics during the past 12 months?	
• Yes	425 (79.7)
• No	108 (20.3)
• Did you get the antibiotic on prescription?	
• Yes	393 (73.7)
• No	140 (26.3)
• When you receive antibiotics, did you always complete the course of antibiotic?	
• Yes	320 (60.0)
• No	69 (12.9)
• Sometimes	144 (27.0)

Participants were asked to describe their behaviors when dealing with antibiotics (Table 3). Results showed that approximately half of the participants visit doctors if they believe they have an infection (n= 241, 45.2%), while about one-fourth (n= 129, 24.2%) reported directly visiting a pharmacy and seeking advice and counseling from the pharmacists. Other participants reported visiting a pharmacy to purchase a previously used antibiotic (n= 60, 11.3%), or asking family members or friends about their experience with an antibiotic they had used previously (n= 19, 3.6%). Only 15.8% reported staying at home without taking antibiotics (n= 84).

Furthermore, participants were asked to report their behaviors if their doctor prescribed an antibiotic with a specific brand name. From this, 31.9% of them (n= 170) stated they would only take that exact brand name, while 59.1% (n= 315) reported that they would not mind taking the same antibiotic under a different brand name (alternative). Few participants (n= 22, 4.1%) reported that they would not mind taking any other antibiotics recommended by the pharmacist, other than the one prescribed.

Table 3. Participants practice in dealing with antibiotics (n= 533)

Parameters	n (%)
If you feel sick (infection disease symptoms), how do you behave? • Visit your doctor and follow his instruction then purchase the prescribed antibiotic. • Go to the pharmacy directly and ask the pharmacist for his advice and counseling. • Go to the pharmacy and purchase your previously used antibiotic. • Ask your family member or friend about his experience in antibiotic he used previously. • Stay at home without taking antibiotic.	 241 (45.2) 129 (24.2) 60 (11.3) 19 (3.6) 84 (15.8)
If your doctor prescribed an antibiotic with a specific brand name, which of the following describes you best? • I believe to take only the same brand name. • I do not mind taking the same antibiotic (API) with different brand name (alternative) • I don't mind taking any other antibiotics recommended by pharmacist other than the prescribed one. • I don't believe in taking any antibiotic. • Others	 170 (31.9) 315 (59.1) 22 (4.1) 21 (3.9) 5 (0.9)

All participants responded to 12 statements aimed at evaluating their knowledge of antibiotics (Table 4). The results showed that participants demonstrated an overall satisfactory level of knowledge about antibiotics, with an average knowledge score of 9. Approximately 80% of the participants (n= 427) understood that antibiotics can treat bacterial infections, while approximately half of them (n= 292, 54.8%) mistakenly believed that antibiotics can also treat viral infections.

Nearly three-quarters recognized that frequent usage of antibiotics can lead to the emergence of resistant bacterial strains. However, the majority of participants believed they could use leftover antibiotics in the future (n= 375, 70.4%), and an even larger percentage (n= 451, 84.6%) believed one could stop taking the medication once they feel better.

Furthermore, 82.2% of the participants (n= 438) reported believing that receiving antibiotics several times would diminish their body's immunity, and a similar percentage (n= 460, 86.4%) believed antibiotics could predispose them to an allergic reaction. Additionally, approximately 70.4% (n=375) reported using antibiotics for bone and body pain.

Table 4. Participants' knowledge about antibiotics (n= 533)

Statements	Correct answers n (%)
Antibiotics are medicines that can treat bacterial infections [a]	427 (80.1)
Antibiotics can be used to treat viral infections [b]	292 (54.8)
Antibiotics are used always in flu and cold symptoms [b]	330 (61.9)
Antibiotics can cure all infections [b]	362 (67.9)
Antibiotics are used to relieve body pain and bone pain [b]	375 (70.4)
Antibiotics kill the transient (bad) bacteria that cause the infection, and it has no effect on normal flora[b]	262 (49.2)
Receiving antibiotics several times will reduce the body immunity [a]	438 (82.2)
Leftover antibiotics can be saved for future use [b]	375 (70.4)
If one feels better after only partially completing an antibiotic course, one can terminate the therapy immediately[b]	451 (84.6)
Antibiotics can cause allergic reaction for some people [a]	460 (86.4)
Frequent usage of antibiotics leads to development of resistant strains of bacteria [a]	407 (76.4)
Suspension dosage form of antibiotic should be stored at refrigerator temp (3-8C) after reconstitution[a]	318 (59.7)
Knowledge score (out of 12.0): median (IQR)	9.0 (5.0)

IQR: Interquartile range. a: True statement. b: False statement

Concerning the sources of information about antibiotics (Figure 1), the main sources reportedly used by the study participants were healthcare providers (physicians and pharmacists) (n= 336, 63.0%), medical websites (n= 182, 34.1%), and academic societies (n= 456, 26.6%). The least commonly selected sources were social media (n= 81, 15.2%), and television (n= 40, 7.5%). Surprisingly, 4.7% of the participants (n= 25) reported that they were not interested in knowing anything about antibiotics.

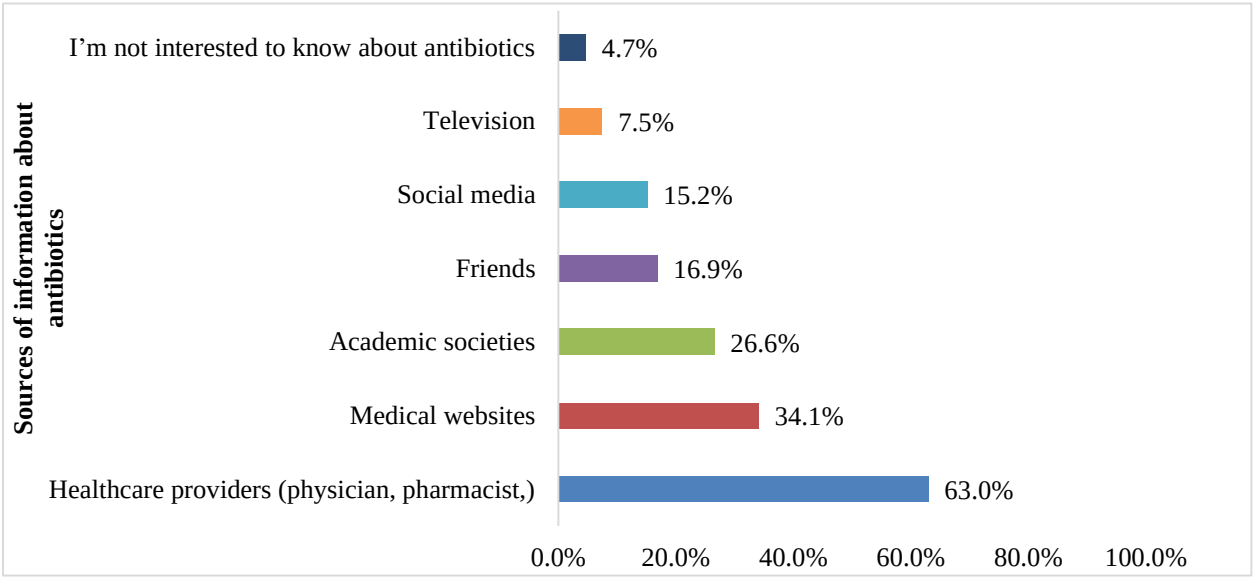

Figure 1. Sources of information about antibiotics as reported by the study participants (n= 533)

Participants were asked about their perceptions of antibiotics (Table 5). The results showed that there is significant trust in the decisions made by physicians and pharmacists in Jordan regarding the prescription and dispensation of antibiotics to patients. However, only 36.4% of the participants (n=194) believed that there are fully activated laws to control the dispensing of these medications.

A high percentage of participants (n= 456, 85.6%) believed that there is a lack of antibiotic awareness in Jordan, and the majority (n=485, 91.0%) support the integration of antibiotic awareness at the school level. Furthermore, only a minority of participants (n= 112, 21%) agreed or strongly agreed that social media was a trusted source of information about antibiotics.

Around 68% of respondents (n= 362) believed that a prescription is needed to obtain an antibiotic from pharmacies. Additionally, about three-quarters (n= 395, 74.1%) disagreed with the concept of purchasing antibiotics online.

The majority of participants (n= 365, 68.5%) noted that pharmacists take their time to counsel them and provide information on how to use antibiotics, and 55% (n= 293) agreed or strongly agreed that pharmacists emphasize that bacterial resistance is a consequence of antibiotic misuse.

Table 5. Participants' perceptions towards antibiotics (n= 533)

Statements	Participant' responses n (%)		
	Strongly agree/agree	**Neutral**	**Strongly disagree/disagree**
I think I need a prescription to get an antibiotic from pharmacy in Jordan	362 (67.9)	76 (14.3)	95 (17.8)
Antibiotics should be allowed to purchase via online without prescription	65 (12.2)	73 (15.6)	395 (74.1)
I think there are laws in Jordan to control the dispensing of antibiotics and are fully activated	194 (36.4)	150 (28.1)	189 (35.6)
People should only take antibiotics if recommended by doctors or pharmacist	475 (89.1)	41 (7.7)	17 (3.2)
Pharmacists take their time to inform me on how antibiotics should be used when prescribed	365 (68.5)	107 (20.1)	61 (21.4)
Pharmacist informs the patient how the misuse of antibiotics may lead to development bacterial resistance	293 (55.0)	117 (22.0)	123 (23.0)
There is a lack of antibiotic awareness among the Jordanian population	456 (85.6)	48 (9.0)	29 (5.4)
Spreading awareness of antibiotics should be integrated within the elementary and secondary education	485 (91.0)	32 (6.0)	16 (3.0)
Social media is a trusted source to get information about antibiotics	112 (21.0)	140 (26.3)	281 (52.7)
I am confident in a doctor's/pharmacist's decision if she/he does not recommend antibiotics.	459 (86.1)	56 (10.5)	18 (3.4)

Finally, a linear regression analysis (Table 6) revealed that participants with greater age, female participants, those with higher education, non-insured participants, and those with a degree related to the medical field demonstrated a better understanding of antibiotics compared to others ($P<0.05$).

Table 6. Assessment of factors associated with participants' knowledge about antibiotics.

Parameter	Knowledge score			
	Beta	**P-value [a]**	**Beta**	**P-value [b]**
Age (years)	0.076	0.081[c]	0.148	0.003[d]
Gender • Male • Female	Reference 0.171	<0.001[c]	0.129	0.001[d]
Educational level • Diploma or below • University graduate or above	Reference 0.179	<0.001[c]	0.142	<0.001[d]
Marital status • Married • Others (Single, divorced, or widowed)	Reference -0.052	0.229[c]	-0.047	0.345
Medical insurance • No • Yes	Reference -0.102	0.018[c]	-0.115	0.003[d]
Do you have medical-related degree? • No • Yes	Reference 0.421	<0.001[c]	0.393	<0.001[d]

a: using simple linear regression, b: using multiple linear regression, c: eligible for entry in multiple linear regression, d: significant at 0.05 significance level.

DISCUSSION

The awareness about antibiotic use is still lacking among the Jordanian population (14,15,16), as in several countries around the world (17,18). Despite efforts to illustrate how antibiotics should be handled and the detrimental consequences of self-medication with antibiotics on bacterial resistance (19,20) and reduction of body immunity (20) by impacting the normal flora, people continue to self-diagnose and self-prescribe these medications, as well as misuse them (5,6,14).

Our study aimed to gather data showcasing the knowledge and perceptions regarding antibiotic usage among the Jordanian population. It examined misconceptions about when and how these medications should be used, practices of self-prescription, and trust in the decisions made by healthcare workers concerning the need to dispense such drugs. This evaluation of the Jordanian community follows many literature sources identifying the deficit in antibiotic knowledge and recommending the institution of campaigns and workshops to raise awareness about these agents (5,6,14).

Our results showed an acceptable level of knowledge about antibiotics. For example, 80% of the participants believe that antibiotics are used to treat bacterial infections, versus 60% as reported by a previous study in Jordan in 2020, that only included parents (thus only married participants), and fewer participants with medically related degrees compared to our study. This may

partially explain the difference in knowledge (5). However, the lack of knowledge was still apparent in some areas. For instance, about two-thirds of respondents agreed that antibiotics are useful when they have flu or cold symptoms, a slightly higher percentage than reported by other studies around the world (21,22). We found that around 78% of participants have received antibiotics at least once in the past 12 months, which is about twice the high percentage among other nations, such as Polish adults (38%) within the same period (23).

Furthermore, the study showed that 55% of participants are informed by pharmacists that antibiotic resistance can develop due to misuse of these drugs. In contrast, a Cypriot study found pharmacists were more actively involved in educating their patients (72.3%) (24). Our study also indicated that nearly three-quarters of respondents believed that leftover antibiotics can be saved for future infections, similar to findings in a recent study done in Palestine (25). This can be an alarming finding, as access to antibiotics without prescription and the use of leftover antibiotics are major contributors to bacterial resistance (26,27).

As part of further assessing participants' understanding of antibiotic indications, and the impact of their inappropriate use on both immunity and resistance, we found that 67.9% (n=362) of the participants believe antibiotics can cure all infections. Also, 70.4% (n=375) consider using them for bone and body pain, a finding that is also common among university students in Jordan [28]. Additionally, we found that around half of the respondents believe antibiotics only impact or kill harmful bacteria that cause disease, with no effect on normal microflora. These results indicate significant misconceptions that are widely accepted by the population.

The trust in decisions made by physicians and pharmacists regarding the need for antibiotics (86.1%), along with them being the main source for antibiotics-related knowledge (63%), indicates that healthcare teams are highly regarded and referenced for medical inquiries. In contrast, social media, which serves as a source of knowledge for only 15.2% of the participants. Focusing on the role of the pharmacist as a healthcare provider in community pharmacies, the responses of the participants confirm that pharmacists play an essential role not only in advising them on the proper administration of the medication, but also in warning them that misuse of the drug -- whether by stopping it before completing the course, using leftovers, or other practices -- may reduce the efficacy of these agents in future uses due to resistance.

Based on the common practice of prescribing and selling antibiotics in Jordan, trade (brand) names rather than generic names are most often used. In this context, around a third of the respondents stated that they would only take the same brand name, while around 60% reported that they would not mind taking the same antibiotic but with a different brand name (alternative) recommended by a pharmacist.

A significant portion of the participants believes there is a lack of awareness about antibiotics among the Jordanian population (85.6%), and they also advocate for the integration of such knowledge early on in school education (91%). In addition, our study found that those participants with higher levels of education (university graduates and above), medically insured participants, and those who hold a medical-related degree demonstrated significantly higher knowledge scores compared to others.

CONCLUSION

Overall, there was an acceptable level of knowledge about the correct ways to use antibiotics. Given the causative relationship between bacterial resistance and the improper handling of antibiotic medications, such as stopping the medication early in the course and using leftovers, major changes need to occur within the Jordanian community. These efforts should rely on the trust people have in healthcare professionals who play a significant role in guiding patients towards proper practices. However, this effort must go hand in hand with enforcing the current laws

regarding antibiotic dispensing and administration by health authorities such as the Jordan Food and Drug Administration (JFDA) and the Ministry of Health.

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Conflict of interest

The Authors declare that there is no conflict of interest.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

REFERENCES

1. FDA. Food and drug administration: Antibiotics and antibiotic resistance. Learn when to use antibiotics to prevent antibiotic resistance. 2019.
2. Aminov R. I. A brief history of the antibiotic era: lessons learned and challenges for the future. *Frontiers in microbiology*. 2010; 1:134.
3. Cagri Buke A., Ermertcan S., Hosgor-Limoncu M., Ciceklioglu M., Eren S. Rational antibiotic use and academic staff. *International Journal of Antimicrobial Agents*. 2003; 21(1):63-6.
4. Llor C., Cots J. M. Dispensing of antibiotics without a medical prescription and communication skills of pharmacists. *J Atención Primaria*. 2013; 45(9):496.
5. Mukattash T. L., Alkhatatbeh M. J., Andrawos S., Jarab A. S., AbuFarha R. K., Nusair M. B. Parental self-medication of antibiotics for children in Jordan. *Journal of Pharmaceutical Health Services Research*. 2020; 11(1):75-80.
6. Wazaify M., Abood E., Tahaineh L., Albsoul-Younes A. Jordanian community pharmacists' experience regarding prescription and nonprescription drug abuse and misuse in Jordan–An update. *Journal of Substance Use*. 2017; 22(5):463-8.
7. Al-Taani G. M., Al-Azzam S., Karasneh R. A., Sadeq A. S., Mazrouei N. A., Bond S. E., et al. Pharmacists’ Knowledge, Attitudes, Behaviors and Information Sources on Antibiotic Use and Resistance in Jordan. 2022; 11(2):175.
8. Dar-Odeh N. S., Abu-Hammad O. A., Khraisat A. S., El Maaytah M. A., Shehabi A. An Analysis of Therapeutic, Adult Antibiotic Prescriptions Issued by Dental Practitioners in Jordan. *Chemotherapy*. 2008; 54(1):17-22.
9. Abdel-Qader D. H., Albassam A., Ismael N. S., El-Shara A. A., Shehri A., Almutairi F. S., et al. Awareness of Antibiotic Use and Resistance in Jordanian Community. *Journal of Primary Care & Community Health*. 2020; 11:2150132720961255.
10. Yusef D., Babaa A. I., Bashaireh A. Z., Al-Bawayeh H. H., Al-Rijjal K., Nedal M., et al. Knowledge, practices & attitude toward antibiotics use and bacterial resistance in Jordan: a cross-sectional study. *Infection, disease & health*. 2018; 23(1):33-40.
11. Abu Farha R., Suyagh M., Alsakran L., Alsous M., Alefishat E. Parental views of antibiotic use in children with upper respiratory tract infections in Jordan. *Tropical Journal of Pharmaceutical Research*. 2016; 15(9):2009-16.
12. Tabachnick B. G., Fidell L. S. Using Multivariate Statistics (5th Edition): Allyn \& Bacon, Inc.; 2006.
13. World Medical A. World medical association declaration of helsinki: Ethical principles for medical research involving human subjects. *JAMA*. 2013; 310(20):2191-4.

14. Al-Azzam S. I., Al-Husein B. A., Alzoubi F., Masadeh M. M., Al-Horani S. Self-medication with antibiotics in Jordanian population. International Journal of Occupational Medicine Environmental Health. 2007; 20(4):373.
15. Alsayed A. R., El Hajji F. D., Al-Najjar M. A., Abazid H., Al-Dulaimi A. Patterns of antibiotic use, knowledge, and perceptions among different population categories: A comprehensive study based in Arabic countries. *Saudi Pharmaceutical Journal*. 2022; 30(3):317-28.
16. Shehadeh M., Suaifan G., Darwish R. M., Wazaify M., Zaru L., Alja'fari S. Knowledge, attitudes and behavior regarding antibiotics use and misuse among adults in the community of Jordan. A pilot study. *Saudi Pharmaceutical Journal*. 2012; 20(2):125-33.
17. Figueiras A., Caamano F., Gestal-Otero J. J. Sociodemographic factors related to self-medication in Spain. *European Journal of Epidemiology*. 2000; 16(1):19-26.
18. Fuentes Albarrán K., Villa Zapata L. Analysis and quantification of self-medication patterns of customers in community pharmacies in southern Chile. *Pharmacy world science*. 2008; 30(6):863-8.
19. Haddadin R. N., Saleh S. A., Ayyash M. A., Collier P. J. Occupational exposure of pharmaceutical workers to drug actives and excipients and their effect on Staphylococcus spp. nasal carriage and antibiotic resistance. *International Journal of Occupational Environmental Health*. 2013; 19(3):207-14.
20. Zhang S., Chen D.-C. Facing a new challenge: the adverse effects of antibiotics on gut microbiota and host immunity. *Chinese Medical Journal*. 2019; 132(10):1135-8.
21. Effah C. Y., Amoah A. N., Liu H., Agboyibor C., Miao L., Wang J., et al. A population-base survey on knowledge, attitude and awareness of the general public on antibiotic use and resistance. *Antimicrobial Resistance Infection Control*. 2020; 9(1):1-9.
22. Kong L. S., Islahudin F., Muthupalaniappen L., Chong W. W. Knowledge and expectations on antibiotic use among older adults in Malaysia: A cross-sectional survey. *Geriatrics*. 2019; 4(4):61.
23. Mazińska B., Strużycka I., Hryniewicz W. Surveys of public knowledge and attitudes with regard to antibiotics in Poland: Did the European Antibiotic Awareness Day campaigns change attitudes? *PloS one*. 2017; 12(2):e0172146.
24. Michaelidou M., Karageorgos S. A., Tsioutis C. Antibiotic use and antibiotic resistance: Public awareness survey in the Republic of Cyprus. *Antibiotics*. 2020; 9(11):759.
25. Hejaz HA. Knowledge and Attitudes towards Antibiotic Usage. *Jordan j. pharm. sci*. 2023; 16(2):447.
26. Machowska A., Stålsby Lundborg C. Drivers of irrational use of antibiotics in Europe. *International Journal of Environmental Research and Public Health*. 2019; 16(1):27.
27. Antwi A. N., Stewart A., Crosbie M. Fighting antibiotic resistance: a narrative review of public knowledge, attitudes, and perceptions of antibiotics use. *Perspectives in Public Health*. 2020; 140(6):338-50.
28. Matalqah LM, Albals D, Radaideh KM, Al-Khateeb H, Thabet RH, Abu-Ismail L, Abunasser M. Knowledge, Attitudes and Practice toward Antibiotic Use among Under and Post-Graduate Students at Yarmouk University in Jordan: A Descriptive Study. *Jordan j. pharm. sci*. 2022; 15(3):378-89.

أنماط معرفة وتصورات واستخدام المضادات الحيوية في المجتمع الأردني: دراسة مقطعية

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ملخص

الخلفية: الممارسات المتعلقة باستخدام المضادات الحيوية لها آثار ضارة على مناعة الجسم، ومقاومة البكتيريا، والصحة العامة للسكان. تؤدي المفاهيم الخاطئة المتعلقة باستخدام المضادات الحيوية إلى سوء الاستخدام وسوء التصرف، مما يشكل تهديدات صحية عديدة.

الأهداف: تهدف هذه الدراسة إلى تقييم معرفة وممارسات ووعي البالغين الأردنيين بالمضادات الحيوية وفهمهم لاستخدامات المضادات الحيوية ومصادر المعرفة.

أسلوب البحث: تمت دعوة عينة كافية من البالغين الأردنيين لملء استطلاع عبر الإنترنت يتم توزيعه عبر منصات التواصل الاجتماعي.

النتائج الرئيسية: أظهرت النتائج أن 77.6٪ من المشاركين قد تلقوا مضادًا حيويًا مرة واحدة على الأقل في آخر 12 شهرًا. أفاد حوالي الثلثين أنهم لا يواجهون مشكلة في الحصول على اسم علامة تجارية مختلف (بديل) للمضاد الحيوي الموصوف. غالبية المستجيبين يثقون في القرار الذي اتخذه الصيادلة. أيضًا، أظهر المشاركون ذوو الأعمار الأعلى، والمشاركات الإناث، وذوو التعليم العالي، والمشاركين غير المؤمن عليهم، وذوي الدرجات الطبية ذات الصلة معرفة أفضل بالمضادات الحيوية مقارنة بالآخرين بدرجة ثقة أقل من 0.05 للجميع.

الخلاصة: يوجد مستوى جيد من المعرفة المتعلقة بالمضادات الحيوية بين البالغين الأردنيين. ومع ذلك، فإن المفاهيم الخاطئة والاستخدامات غير الصحيحة منتشرة أيضًا، ويجب تركيز الانتباه على تصحيح مثل هذه الممارسات التي تؤثر على الصحة العامة للمجتمع.

الكلمات الدالة: وعي؛ تصور؛ مضادات حيوية؛ استخدام؛ الأردن.

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Community Pharmacists' Perceptions of the most Important Interventions Implemented in Supporting Breastfeeding Women During Maternal Life: A Cross-Sectional Study in Jordan

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ABSTRACT

Objectives: The purpose of this cross-sectional study is to investigate and analyze community pharmacists' perspectives on the most essential interventions implemented to support breastfeeding women during their maternal life in Jordan.

Methods: A cross-sectional study design was employed using a self-administered survey. A convenience sample (n = 381) of community pharmacists was recruited via social media resources. Responses were subject to various statistical analyses using SPSS.

Key Findings: In the study with 381 participants, the majority were females (86.4%, n=329) aged between 23 and 30 (78.7%, n=300). A total of 55.1% (n=210) had previous breastfeeding experience. Pharmacists (65.9%, n=251) stressed the significance of health education for improved breastfeeding support. During prenatal care, 42% (n=160) underscored the importance of engaging parents, while 37% (n=141) highlighted the need for additional lactation support during the delivery stage. Finally, 34.4% (n=131) identified serving as an informational resource for the "mother-baby dyad" as being crucial in the postnatal stage.

Conclusions: The study spotlights the critical role of pharmacists in breastfeeding support, underscoring the importance of tailored interventions based upon their demographics and perceptions. These insights provide invaluable guidance for optimizing community pharmacists' contributions to maternal care.

Keywords: Pharmacist, Breastfeeding, Perception, Support, Education, Jordan.

INTRODUCTION

In 1981, Bo Vahlquist emphasized that the reproductive cycle of all mammalian species, including humans, encompasses both pregnancy and breastfeeding, highlighting the latter's pivotal role in survival (1). The prevalent consensus on optimal infant nutrition underscores the presiding adage, 'breast is best' (2). A 2016 Lancet series projected that the implementation of optimal breastfeeding practices could annually prevent 823,000 deaths among children under five years of age (3). Despite

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burgeoning interest in breastfeeding and plenty of scientific evidence supporting its importance, the global rates of exclusive breastfeeding for infants younger than six months remain disappointingly low at 44% (4).

Jordan stands out due to its high prevalence of breastfeeding, with 92% of children experiencing nursing at some point(5). However, previous studies in Jordan have revealed unfavorable attitudes towards breastfeeding practices. Moreover, the rate of exclusive breastfeeding among six-month-old babies is less than ideal and has been declining, decreasing from 40% in 2007 to 26% in 2018, according to Jordanian population and family health surveys(5).

The global imperative to enhance breastfeeding practices was underscored by its inclusion in the Millennium Development Goals outlined by the World Health Organization (WHO) over the past decade (6). Despite this, evidence suggests that many new mothers struggle to meet their desired infant-feeding goals, emphasizing the need for breastfeeding support (7). While some women face medical or physical barriers that inhibit breastfeeding, many others can benefit from adequate support. In 2011, the US Surgeon General issued a Call to Action to Support Breastfeeding, urging communities, families, and healthcare professionals to facilitate breastfeeding for mothers (8). That same year, the Council of the International Pharmaceutical Federation (FIP) endorsed a paper highlighting the essential role pharmacists can play in improving maternal, neonatal, and child health (9). Given their frequent interactions with parents and their knowledge of medication safety, pharmacists are well-positioned to play a crucial role in promoting and supporting breastfeeding (10).

Despite recognizing the potential role of pharmacists in breastfeeding support, literature on their knowledge and education in this area is limited, with existing studies primarily focusing on medicine and lactation knowledge (11-14). Recently, a study in Jordan highlighted the importance of prioritizing patient satisfaction in general pharmacy settings, emphasizing the need to focus on patient contentment when providing pharmaceutical services (15). However, information about pharmacists' perspectives on their interventions during pregnancy in Jordan is scarce. This study aims to investigate community pharmacists' perspectives on the most significant interventions implemented to support breastfeeding mothers during their maternity journey in Jordan.

METHODS

Research Design and Participants

This research employed a descriptive cross-sectional design and utilized an online survey to address its objectives. Conducted in Jordan, the study ran from August 2, 2021, to February 17, 2022. Clinical researchers developed and validated an online survey to gather anonymous responses, ensuring confidentiality. The survey targeted community pharmacists in Jordan who held at least a bachelor's degree or Doctor of Pharmacy and were currently working in community pharmacies. Participants were recruited using convenience sampling through social media platforms like Facebook and WhatsApp groups. Following the inclusion criteria explained at the survey's outset, participants were informed about the voluntary nature of their involvement and that there were no risks. A written consent statement was provided at the beginning of the survey, with participants having the option to either agree to proceed or choose "I disagree to participate" to decline. Completion of the survey represented informed consent. Participant anonymity was maintained, and ethical approval was obtained from the Institutional Review Board of King Abdullah University Hospital, University of Science & Technology, Jordan (Reference No. 56/141/2021).

Survey Development, Validation, and Reliability

The online survey was developed after reviewing

validated surveys in the literature and scrutinized by a focus group of pharmacy practice experts. A draft questionnaire was then pilot-tested with 10 community pharmacists for comprehension, clarity, and cultural acceptability before the main survey. Administered via Google Forms, the survey was self-administered in English and designed to be completed within 10–12 minutes.

Sample Size

With 22,667 registered pharmacists in Jordan as of February 2019, the Raosoft® calculator recommended a sample size of 378 participants for a 5% margin of error and a 95% confidence level. To account for potential unknown issues, the sample size was increased to approximately 380 pharmacists.

Statistical Analyses

Completed surveys were extracted from Google Forms and transferred to SPSS version 25.0 for analysis. Categorical variables were presented as frequencies or percentages.

RESULTS

Sociodemographic characteristics

Upon completion of the online questionnaire distribution, a total of 381 fully completed forms were incorporated into the study. Predominantly, the demographic profile of participants leaned towards females, constituting 86.4% (n=329), with the majority falling within the 23-30 age group, which accounted for 78.7% (n=300). An exploration of marital status revealed that a significant portion, 58% (n=221), identified as single, while nearly 70% (n=265) reported not having any children. Notably, over half the study sample, specifically 55.1% (n=210), possessed personal experience with breastfeeding. For a comprehensive overview of the participants' demographic characteristics, refer to Table 1.

Table 1. Demographic characteristic of the study participants and their principal place of practice

Characteristics	n, (%)
Age	
23 – 30 years	300, (78.7)
31 – 40 years	55, (14.4)
41 – 50 years	22, (5.8)
More than 50 years	4, (1.0)
Gender	
Female	329, (86.4)
Male	52, (13.6)
Marital status	
Single	221, (58.0)
Married	152, (39.9)
Divorced	7, (1.8)
Widow	1, (0.3)
Do you have any children?	
Yes	116, (30.4)
No	265, (69.6)
Years of practices	
Less than 5 years	286, (75.1)
Between 5 and 10 years	48, (12.6)
Between 11 and 20 years	33, (8.7)
More than 20 years	14, (3.7)
Highest degree	
BSc	234, (61.4)
Pharm D	96, (25.2)
MSc	39, (10.2)
PhD	12, (3.1)
Geographical area of practice	
North of Jordan	165, (43.3)
Middle of Jordan	197, (51.7)
South of Jordan	19, (5.0)
Source of highest degree	
Jordan	375, (98.4)
Others country	6, (1.6)
Work Location	
Rural (Village)	86, (22.6)
Urban (City)	295, (77.4)
Pharmacy type	
Independent	280, (73.5)
Small chain < = 20 branches	71, (18.6)
Big chain > 20 branches	30, (7.9)
Do you have personal experience in breastfeeding?*	
Yes	210, (55.1)
No	171, (44.9)

*For the purposes of this survey, personal experience in breastfeeding is defined as having breastfed yourself; or having a spouse/partner (wife) who has breastfed; or have a family member who has breastfed, with whom you have spent significant time, including overnight, while they were breastfeeding.

Pharmacists' Perception in Supporting Breastfeeding during all Maternal Life

Table 2 illustrates the frequency distribution of responses from surveyed pharmacists as they identify key factors influencing their inclination to provide more support for breastfeeding across various stages of maternal life. A significant majority, comprising 65.9% (n=251), expressed the belief that conveying education on the important health benefits associated with breastfeeding, in accordance with the best standards of care, is a pivotal intervention for offering enhanced support during the pre-pregnancy stage. For the prenatal stage, 42% (n=160) of pharmacists indicated that engaging parents in discussions about feeding, given the role of pharmacies as primary suppliers of various baby products, can significantly influence their support for breastfeeding. Conversely, during the delivery stage, 37% (n=141) perceived that offering additional coverage for lactation-related queries when other nurses and lactation consultants are unavailable is the most influential factor. Furthermore, in the postnatal period, over one third of pharmacists (34.4%, n=131) identified serving as an information resource for the "mother–baby dyad" as the intervention that enhances their role as better supporters of breastfeeding.

Table 2. Pharmacists' perception about the most important intervention in supporting breastfeeding during all maternal life.

Pre-pregnancy		
• Provide education about the importance of the health benefits associated with breastfeeding according to the best standard of care.	**N**	**251**
	%	**65.9**
• Provide patient support through advocacy of prenatal vitamins and documentation of checklists of contraindicated medications/ supplements during pregnancy.	**N**	**130**
	%	**34.1**
Prenatal		
• Engage parents in discussions about feeding (pharmacies are a primary supplier of a wide range of baby products including breast pads, nursing cover-ups, baby carriers or slings, breast pumps and pumping supplies, nipple shields, ointments for breastfeeding, diapers, toys, bibs, and baby bath and skin care products).	**N**	**160**
	%	**42%**
• For parents who have not yet decided on the method they would like to use to feed their infants, pharmacists can help with prenatal education around breastfeeding and formula feeding.	**N**	**115**
	%	**30.2%**
• Provide information about the normal course of breastfeeding, the management of common difficulties, and considerations for preparing for breastfeeding.	**N**	**97**
	%	**25.5%**
• Direct parents to available support groups.	**N**	**9**
	%	**2.4%**
Delivery		
• Provide additional coverage on lactation-related questions when other nurses and lactation consultants are not available due to high inpatient census or absence of 24/7 coverage.	**N**	**141**
	%	**37%**
• Provide information on appropriate use of infant formula.	**N**	**115**
	%	**30.2%**
• Provide useful resources of information and skills on how to start and continue the process breastfeed babies.	**N**	**125**
	%	**32.8%**
Postnatal		
• Serve as an information resource for the "mother–baby dyad" (most health care during the postpartum period is directed at either the mother or the infant).	**N**	**131**
	%	**34.4%**
• Provide advice regarding breastfeeding concerns and difficulties (including appropriate referral to other health care professionals and referral to available support groups).	**N**	**127**
	%	**33.3%**
• Provide counseling and education related to pumping and pumping equipment and appropriate storing of breast milk.	**N**	**77**
	%	**20.2%**
• Provide counseling and education related to preparation and use of infant formula.	**N**	**46**
	%	**12.1%**

DISCUSSION

The findings from our study contribute to the ongoing discourse on community pharmacists' attitudes and practices related to breastfeeding support, offering insights into the evolving landscape of pharmacist involvement in breastfeeding support. As the inaugural study in this domain, our research holds a distinctive position, as it pioneers the examination of pharmacists' perspectives on the most crucial interventions supporting breastfeeding women during their maternal life in Jordan. While existing literature provides foundational knowledge on various aspects of maternal care, the absence of prior studies specifically addressing this aspect underscores the novelty and significance of our investigation. Our study, which includes a substantial representation of female participants (86.4%), aligns with the gender distribution observed in previous research within the pharmacy profession. This consistency emphasizes the continued importance of understanding and addressing gender-specific factors in the context of breastfeeding support. Similarly, the concentration of participants within the 23 to 30 age bracket reflects broader trends in the healthcare workforce, suggesting a young and potentially dynamic cohort.

A study conducted by Roger Edwards from the Department of Pharmacy Practice, Northeastern University, Boston, showed that pharmacists have numerous opportunities to educate the general public and parents about ideal infant feeding throughout pre-pregnancy, pregnancy, delivery, and postpartum periods. To understand potential roles for pharmacists in supporting breastfeeding, the study delineated pharmacist interventions into four durations: pre-pregnancy, pregnancy, delivery, and postpartum (10). The significant percentage (55.1%) of pharmacists with personal breastfeeding experience in our study echoes findings from some previous investigations. However, it also underscores the importance of considering personal experiences in the development of supportive attitudes. Interestingly, the high proportion of participants without children (70%) introduces a distinctive feature not extensively explored in prior studies. This demographic characteristic warrants further investigation to understand its potential impact on the support practices of pharmacists.

When comparing our results on perceptions and interventions with existing literature, both congruencies and disparities emerge. The emphasis on education regarding the health benefits of breastfeeding, identified by 65.9% of pharmacists in our study during the pre-pregnancy stage, aligns with the emphasis on knowledge dissemination found in some previous research. However, the nuanced focus on engaging parents in discussions about feeding during the prenatal stage (42%) adds a unique dimension to the understanding of pharmacists' roles, particularly given their status as primary suppliers of infant products.

The identification of lactation-related query coverage as a significant factor during the delivery stage (37%) resonates with studies emphasizing the importance of timely and accessible information. Conversely, the emphasis on serving as an information resource for the "mother–baby dyad" during the postnatal period (34.4%) introduces a broader, holistic approach not extensively explored in previous literature.

Our study aligns with the global imperative to improve breastfeeding practices, as highlighted by the WHO's Millennium Development Goals. The U.S. Surgeon General's Call to Action in 2011 and the subsequent endorsement by the International Pharmaceutical Federation (FIP) underscore the international recognition of pharmacists' potential in promoting breastfeeding (9).

Practicing pharmacists need support now, and changes to undergraduate education are warranted to keep pace with current developments and changes in practice (16). Notably, our study fills a significant gap in the literature by focusing solely on pharmacists' perspectives on the most important interventions implemented to support breastfeeding mothers in Jordan during their maternity years. Our goal is not only to add substantial knowledge to this area, but also to lay the groundwork for enhancing the role of community pharmacists in maternal care by investigating and recording their insights. This study has the potential to improve the general support network for breastfeeding women in the

Jordanian community by informing healthcare policies and developing focused interventions.

CONCLUSIONS

While our findings align with certain patterns identified in previous studies, the unique demographic characteristics and nuanced perceptions uncovered in our investigation highlight the evolving nature of pharmacist roles in breastfeeding support. This study contributes valuable data to the existing body of knowledge and provides a foundation for future research and tailored interventions aimed at optimizing the contributions of community pharmacists in this crucial healthcare domain.

Conflict of Interest Statement

The author(s) declare that there are no conflicts of interest.

Data access statement

All authors had and still have complete access to the study data.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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REFERENCES

1. Organization WH. Contemporary patterns of breast-feeding: report on the WHO Collaborative Study on Breast-feeding: World Health Organization; 1981.
2. Dykes F. Breastfeeding in hospital: mothers, midwives and the production line: Routledge. 2006.
3. Victora CG., Bahl R., Barros AJ., França GV., Horton S., Krasevec J., et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. The Lancet. 2016; 387(10017):475-90.
4. UNICEF. Infant and young child feeding. Adopting optimal feeding practices is fundamental to a child's survival, growth and development, but too few children benefit. UNICEF. 2021.
5. Department of Statistics ICF. Jordan Population and Family Health Survey 2017-18 March 2019 [Available at: https://dhsprogram.com/pubs/pdf/FR346/FR346.pdf.
6. Organization WH. Early initiation of breastfeeding to promote exclusive breastfeeding. World Health Organization www who int/elena/titles/early_breastfeeding/en/,/entity/elena/titles/early_breastfeeding/en/index html. 2019.
7. Declercq E., Labbok MH., Sakala C., O'Hara M. Hospital practices and women's likelihood of fulfilling their intention to exclusively breastfeed. *American Journal of Public Health*. 2009; 99(5):929-35.
8. Health UDo, Services H. The Surgeon General's call to action to support breastfeeding 2011.
9. Partnership for Maternal NCH. International Pharmaceutical Federation report on effective use of pharmacists in MNCH 2012, Feb 23 [Available at: https://www.who.int/pmnch/media/news/2012/20120223_fip_paper/en/.
10. Edwards RA. Pharmacists as an underutilized resource for improving community-level support of breastfeeding. *Journal of Human Lactation*. 2014; 30(1):14-9.
11. Ronai C., Taylor JS., Dugan E., Feller E. The identifying and counseling of breastfeeding women by pharmacists. *Breastfeeding Medicine*. 2009; 4(2):91-5.
12. Merlob P., Stahl B., Kaplan B. Drug use in pregnancy and breast feeding: the role of the pharmacist. *International Journal of Risk & Safety in Medicine*. 1998; 11(1):45-7.
13. Akus M., Bartick M. Lactation safety recommendations and reliability compared in 10 medication resources. *Annals of Pharmacotherapy*. 2007; 41(9):1352-60.
14. Hussainy SY., Dermele N. Knowledge, attitudes and practices of health professionals and women towards medication use in breastfeeding: A review. *International Breastfeeding Journal*. 2011; 6(1):1-16.
15. Amara N., Naser AY., Esra' O. T. Patient Satisfaction with Pharmaceutical Services in Jordan: A Cross-Sectional Study. *Jordan j. pharm. sci*. 2023; 16(1):1-10.
16. Hjazeen R. Community Pharmacists' Perspectives toward Continuing Professional Development: A Qualitative Study. *Jordan j. pharm. sci*. 2023; 16(2):449

تحليل تصورات المجتمع لأهم التدخلات المنفذة في دعم المرأة المرضعة أثناء الحياة الأمومية: دراسة مقطعية في الأردن

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ملخص

الأهداف: الغرض من هذه الدراسة المقطعية هو دراسة وتحليل تصورات صيادلة المجتمع حول أهم التدخلات المنفذة لدعم النساء المرضعات خلال حياتهن الأمومية في الأردن.

الطرق: تم إجراء تصميم دراسة مقطعية باستخدام المسح الذاتي. تم تعيين عينة ملائمة (ن = 381) من صيادلة المجتمع من خلال موارد وسائل التواصل الاجتماعي. خضعت الإجابات لتحليلات إحصائية مختلفة باستخدام برنامج SPSS.

النتائج الرئيسية: شملت الدراسة 381 مشاركًا، معظمهم من الإناث (86.4%، العدد = 329) الذين تتراوح أعمارهم بين 23 إلى 30 عامًا (78.7%، العدد = 300)، 55.1% (العدد = 210) لديهم خبرة في الرضاعة الطبيعية. وشدد الصيادلة (65.9%، العدد = 251) على أهمية التثقيف الصحي لدعم الرضاعة الطبيعية بشكل أفضل. أثناء رعاية ما قبل الولادة، أكد 42% (العدد = 160) على إشراك الوالدين، في حين سلط 37% (العدد = 141) الضوء على التغطية الإضافية للرضاعة في مرحلة الولادة. أخيرًا، حدد 34.4% (العدد = 131) أن العمل كمصدر معلومات لـ "الثنائي الأم –الطفل" أمر بالغ الأهمية بعد الولادة.

الاستنتاجات: تسلط الدراسة الضوء على دور الصيادلة الحاسم في دعم الرضاعة الطبيعية، مع التركيز على أهمية التدخلات المصممة بناء على التركيبة السكانية وتصوراتهم. توفر هذه الأفكار توجيهًا قيمًا لتحسين مساهمات صيادلة المجتمع في رعاية الأمومة.

الكلمات الدالة: صيدلاني، الرضاعة الطبيعية، الإدراك، الدعم، التعليم، الأردن.

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Assessment of Extraction Methods Effects on the Biological Activities (Antioxidant and Antiamylase) and Chemistry (Total Phenolics and Flavonoids) of *Guazuma ulmifolia* Leaves

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ABSTRACT

The antioxidant activity was tested using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method. Antiamylase activity was evaluated through a colorimetric assay that employs 3,5-dinitro salicylic acid (DNSA) as a substrate. Total phenolics and flavonoids content were quantified by the colorimetric method. The highest yield from the extraction of G. ulmifolia leaves was obtained from the water extract (9.64%). The infusion showed the most robust antioxidant and antiamylase activities (IC50 = 6.853 ± 0.504 µg/mL and 261.03 ± 6.83 µg/mL, respectively). The highest total phenolics and flavonoids content were found in the ethanolic extract, with 69.848 ± 1.871 mg GAE/g extract and 118.854 ± 1.001 mg QE/g extract respectively. Total phenolics and flavonoids content significantly influenced the antioxidant activity, but not the antiamylase activity. In conclusion, infusions were the best extraction method for obtaining high antiamylase activity, even though they did not yield the highest total phenolics and flavonoids content. Further research is needed to identify the compound in G. ulmifolia leaf infusions that contribute to antioxidant and antiamylase activities.

Keywords: *Guazuma ulmifolia*, extraction methods, antioxidant, antiamylase, phenol, flavonoids.

INTRODUCTION:

In Indonesia, the leaves of Guazuma ulmifolia, locally known as jati belanda, have been used traditionally for anti-obesity and anti-diabetic treatments [1]. The medicine is prepared by boiling several leaves in water until the volume is reduced to one quarter of the original quantity [2]. Existing research supports the use of G. ulmifolia leaves for anti-diabetic treatment, such as the study conducted by Adnyana et al., which showed that a water leaf extract has an anti-diabetic effect [3]. Similar results were shown by combining G. ulmifolia and Tecoma stans to improve the glycaemic profile in patients with type 2 diabetes mellitus [4]. The anti-diabetic mechanism of G. ulmifolia leaves was identified by Alonso-Castro et al. who found that it stimulates glucose uptake [5]. Another anti-diabetic mechanism is related to inhibiting amylase activity, thereby reducing blood glucose levels due to the limitation of saccharide digestion. Therefore, natural products capable of inhibiting amylase have the potential to be developed as anti-diabetic agents, including polyphenols and flavonoids [6]. A relationship exists between the number of hydroxyl groups on the B ring of polyphenol ligands and amylase inhibition potential. This hydroxyl group forms a hydrogen bond with the catalytic residue of the enzyme binding site [7]. Furthermore,

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flavonoids have specific hydroxyl groups at C7 or C4' and conjugated π bonds, forming hydrogen bonds that stabilise the interaction between inhibitors (flavonoids) and the residue of the enzyme. In addition, polyphenols such as chlorogenic acid have also been reported to possess amylase inhibitor activity through the formation of quinone or lactone structures, or compounds with a 4-oxo-pyrane structure [8].

Extraction is the primary step for obtaining bioactive compounds from plant material with the diversity and quantity of the bioactive compounds in the final extract depending substantially on several factors, including the method and solvent used [9]. Traditionally in Indonesia, G. ulmifolia leaves are boiled with water in a decoction process to produce a product known as "Jamu" [2]. Additionally, according to the Indonesian Pharmacopeia 4th Edition, the infusion of leaves is prepared by boiling with water at 90°C for 15 minutes [10]. Both herbal preparations, jamu, and infusion, are intended for use when fresh. However, for the traditional medicine industry, which prefers extracts in a dry form, these methods are seen as inefficient due to the high cost of evaporating water without damaging bioactive compounds. For this purpose, organic solvents are often used to extract bioactive compounds from plant materials to facilitate the drying process, for instance, ethanol [11].

In this research, we examined the impacts of G. ulmifolia leaf preparation methods, water extraction according to "Jamu" preparation used typically in Indonesia [2], infusion as per the Indonesian Pharmacopeia 4th Edition [10], and ethanolic extraction, which is commonly used by the industry [11], on their antioxidant and antiamylase activities, as well as their phenolics and flavonoids content. A previous study on the antioxidant properties of G. ulmifolia leaves utilized organic solvents, specifically n-hexane and ethyl acetate [9], which are not commonly used for traditional Indonesian medicine preparation. Our study focused on extracts commonly used for Jamu preparation in the community, i.e., water, and the industry, i.e., ethanol, to determine not only their antioxidant properties but also antiamylase activity.

MATERIAL AND METHODS:

Sample preparation

The G. ulmifolia leaves were collected from Meru Betiri National Park in Jember, Indonesia, and transported to the University of Jember under the code of GUMB. A voucher sample was sent to the Purwodadi Botanic Garden, National Research and Innovation Agency of Indonesia, in Purwodadi, East Java, for taxonomical identification. The leaves were selected, washed, and dried before being pulverized into a powder. The G. ulmifolia leaves were then extracted using three different methods: water extraction based on the traditional "Jamu" preparation used in Indonesia [2] (termed as water extract), an infusion according to the Indonesian Pharmacopeia 4th Edition[10] (referred to as infus), and an ethanolic extraction typically used by the industry [11] (commonly known as ethanolic extract).

Water extraction

Water extraction was carried out using the traditional method for "Jamu" preparation [2]. Specifically, a quantity of dried leaf powder equivalent to 20 leaves was boiled with three cups of distilled water (approximately 600 mL) until the final volume was reduced to three-quarters of the original. This was then freeze-dried, and the resulting dry extract was used for further tests. The extract obtained was referred to as the water extract.

Infusion

The infusion followed the Indonesian Pharmacopeia 4th Edition [10]. The process began with 10 grams of dried leaf powder, which was extracted with distilled water at 90°C for 15 minutes. Once cool, it was filtered and freeze-dried. This dry extract, known as infus, was used for the subsequent tests.

Extraction with 70% ethanol

To create the ethanolic extract, 200 grams of G. ulmifolia leaf powder was macerated with 1 liter of 70%

ethanol in distilled water for 24 hours, occasionally stirring during this period, before it was filtered. The residue was then re-macerated twice, with the combined filtrate concentrated using a rotary evaporator to produce a crude, dried ethanol extract [11], referred to as the ethanolic extract.

Antioxidant assay

The antioxidant assay was done using DPPH method[12]. A 0.5 mL of the test solution, i.e., the extract or positive control (quercetin) at every concentration, was added to 0.5 mL of DPPH 50 µg/mL. For the negative control, the test solution was replaced with 0.5 mL of methanol. The mixed solution was incubated in a dark place for 15 minutes before its absorbance was measured using a spectrophotometer at a wavelength of 517 nm.

The absorbance data obtained at each concentration was used to calculate the percentage of DPPH scavenging using Equation 1. A regression equation was plotted from the sample concentration (x) to the percentage of DPPH scavenging (y). The regression equation y = bx + a was used to determine the IC50 value using Equation 2.

$$\text{Amylase inhibition (\%)} = \frac{\text{A DPPH} - \text{A Solution test}}{\text{A DPPH}} \times 100\% \quad \text{(Equation 1)}$$

$$IC_{50} = (50 - a)/b \quad \text{(Equation 2)}$$

Antiamylase assay

Evaluation of the antiamylase activity was executed on sample solutions, i.e., the extract and positive control/acarbose, as well as the negative control (without inhibitor) [13]. Each 100 µL of the solution was added to 30 µL of the enzyme solution and incubated at 25ºC for 15 minutes. A total of 250 µL of the substrate was added, followed by incubation at the same temperature and time. The enzymatic reaction was stopped by heating the mixture for 1 minute. A portion of 160 µL of the solution was taken, and 80 µL of DNSA reagent was added, heated on a hot plate for 5 minutes [13]. After cooling, 720 µL of distilled water was added. A total of 100 µL of each mixture of test solutions was added to 100 µl of distilled water, and the absorbance was measured using a microplate reader at a wavelength of 540 nm.

The absorbance data obtained at each concentration was adjusted by the corresponding blank absorbance to further calculate the inhibition of amylase using Equation 3. A regression equation was made from the series of sample concentrations in the microplate reader (x-axis) with the inhibition value (y-axis). The regression equation y = bx + a was used to find the IC50 value as in Equation 2.

$$\text{Amylase inhibition (\%)} = \frac{\text{A Control (-)} - \text{A Solution test}}{\text{A Control (-)}} \times 100\% \quad \text{(Equation 3)}$$

The linear regression was constructed by plotting the value of amylase inhibition against the extract concentration. This linear regression was used to calculate the IC50 values of the extracts.

Total Phenolics Content Quantification

The total phenolics content was tested by reacting 50 µL of the extract or gallic acid standard with 1.25 mL of Folin Ciocalteu reagent, which was then incubated for 6 minutes. The mixture was supplemented with 1.25 mL of 7% Na2CO3 and then re-incubated for 1 hour. The mixture was subsequently transferred into a cuvette to measure its absorbance at a wavelength of 725 nm [13, 14].

Total Flavonoids Content Quantification

The quantification of total flavonoids content was performed by reacting 150 µL of the sample (either the extract or quercetin as a standard) with 30 µL of 5% NaNO2, 30 µL of AlCl3 10%, and 400 µL of distilled water. The mixture was incubated for 6 minutes before adding 200 µL of 1N NaOH and 240 µL of distilled water. After homogenization, the absorbance of the solution was measured at a wavelength of 415 nm [14].

Statistical Analysis

The results were reported as mean ± standard deviation (SD). The regression method was used to calculate the IC50 of antiamylase and antioxidant activities. Least Significant Difference (LSD) was used to analyze significant differences among the mean values. Multiple regression analysis was used to evaluate the relationship between total phenolic and flavonoids content on antioxidant and antiamylase activity, and the relationship between total phenolic and flavonoids content and antioxidant activity on antiamylase activity. Pearson correlation was used to determine the contribution of total phenolic and flavonoids content to antioxidant and antiamylase activity. Values of $p < 0.01$ were considered significant.

RESULTS AND DISCUSSION:

The yield of the extract

The yields from the water extract, infus, and ethanolic extract of G. ulmifolia leaves are summarized in Figure 1. Extraction yield (mass of extract/mass of dried leaves powder) was used as an indicator of the efficiency of the extraction methods. The results showed that the highest yield was obtained from water extraction (9.64%), followed by infusion (8.96%) and ethanol extraction (5.92%). These results are consistent with previous reports showing that the yield of extraction with water is higher than that of ethanol [15]. The primary metabolites in G. ulmifolia leaves, such as proteins and carbohydrates (xanthan gum), coexist with secondary metabolites but are produced in larger quantities [16]. These primary metabolites are polar and therefore more soluble in water, which may explain why the yield of water extract and infusion was higher than that of the ethanolic extract [15].

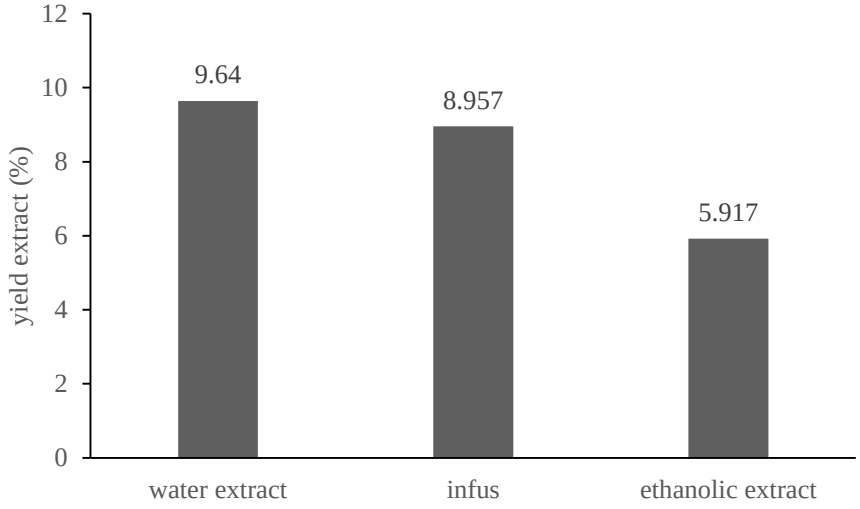

Figure 1. The yield of extract

Antioxidant activity

The results of the antioxidant activity test showed that all three extracts exhibited concentration-dependent antioxidant activity (Figure 2). Although all extracts demonstrated very strong antioxidant activity based on their inhibition of DPPH, infus exhibited the best antioxidant activity (Table 1).

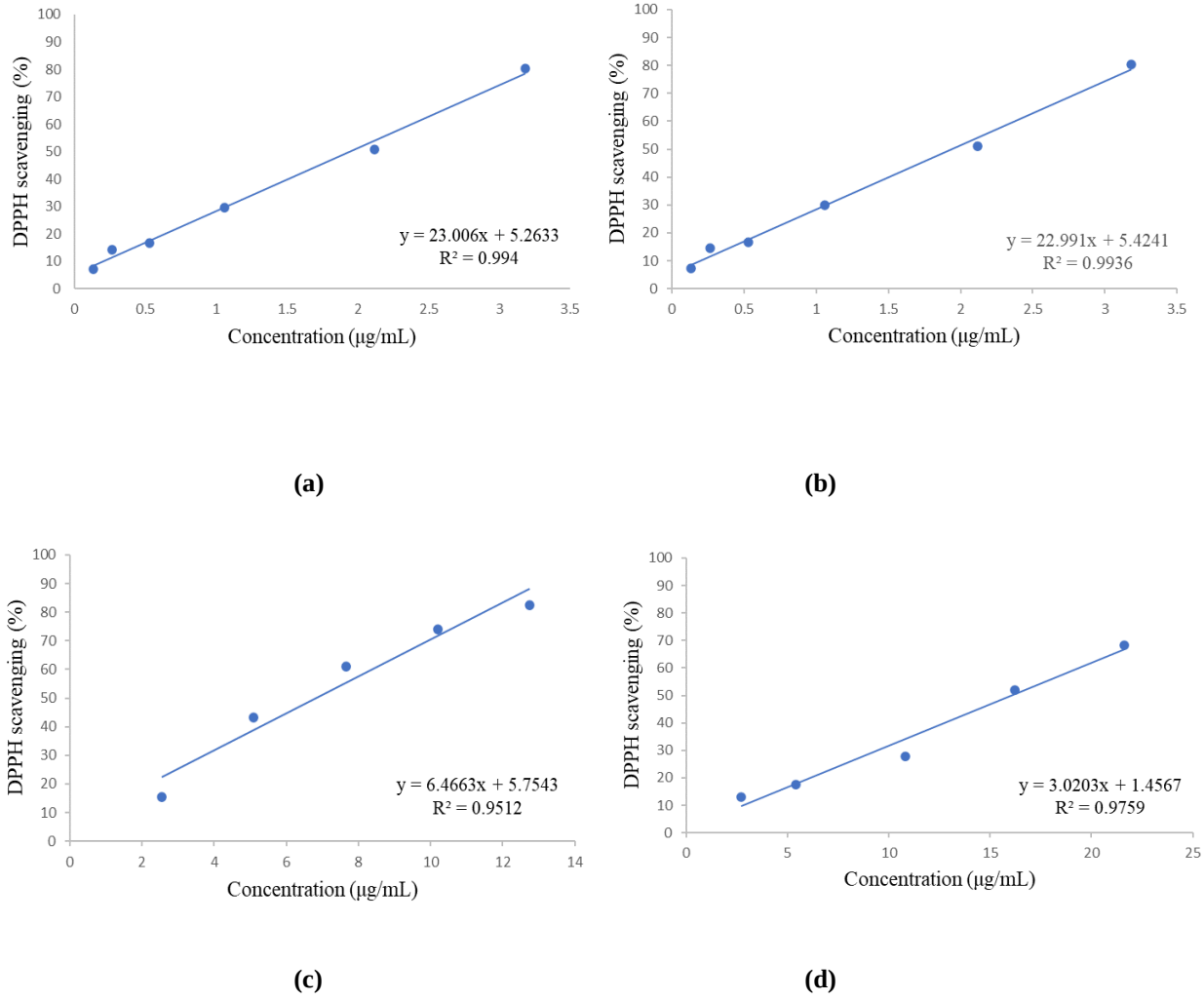

Figure 2.: Correlation concentration of quercetin (a), water extract (b), infus (c), and ethanolic extract (d) of *G. ulmifolia* leaves and DPPH scavenging. All samples are linear, the higher the concentration, the higher the DPPH scavenging activity ($p < 0.01$).

Table 1. IC_{50} of antioxidant activity

Samples	IC_{50} (µg/mL)*	Antioxidant activity category[21]
Quercetin	1,945 ± 0,021[a]	very strong
Water extract of *G. ulmifolia* leaves	9,811 ± 0,805[b]	very strong
Infus of *G. ulmifolia* leaves	6,853 ± 0,504[c]	very strong
Ethanolic extract of *G. ulmifolia* leaves	16.070 ± 0.497[d]	very strong

*Data are presented as means ± SD (n = 3), different notation showed significant difference ($p<0.01$)

Antiamyase activity

The impact of each extract on amylase inhibitory activity was evaluated. The test results indicated that the percentage of enzyme inhibition increased with the concentration of the extract (Figure 3). Although no significant difference was observed between the water extract and ethanolic extract, infus demonstrated the most potent antiamylase activity (Table 2).

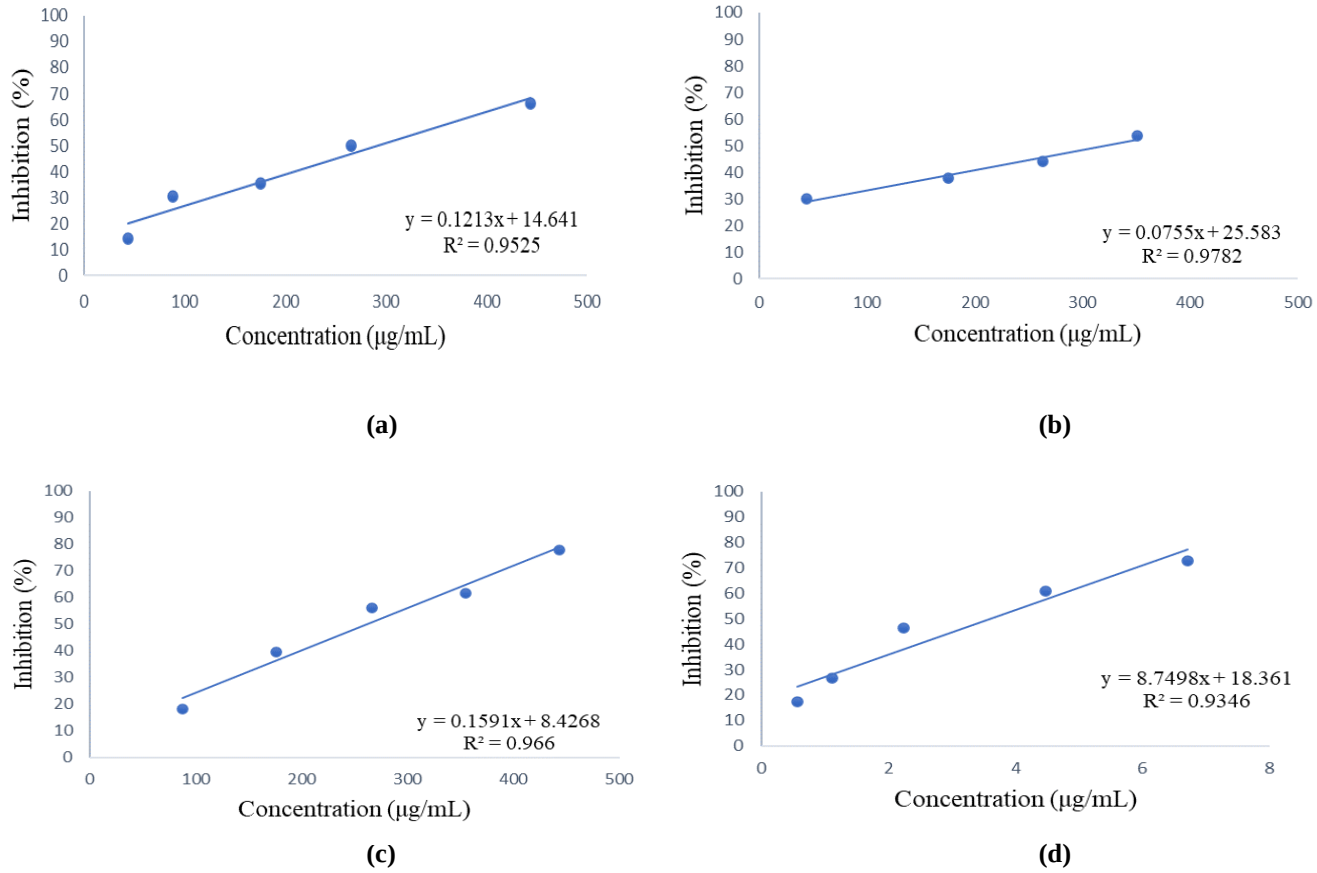

Figure 3. Correlation concentration of acarbose (a), water extract (b), infusion (c), and ethanolic extract (d) of *G. ulmifolia* leaves and α-amylase inhibition. All samples are linear, the higher the concentration, the higher the amylase inhibition activity ($p < 0.01$).

Table 2. IC_{50} of α-amylase inhibitory activity

Samples	IC_{50} (µg/mL)*
Acarbose	3,609 ± 0,383[a]
Water extract of *G. ulmifolia* leaves	318,273 ± 43,514[b]
Infus of *G. ulmifolia* leaves	261,030 ± 6,829[b]
Ethanolic extract of *G. ulmifolia* leaves	291,674 ± 8,205[b]

*Data were presented as means ± SD (n = 3), different notation showed significant difference ($p<0.01$).

Total Phenolics Content

The total phenolics content of the extracts was calculated from the gallic acid standard curve using the correlation equation y = 0.1732x + 0.0112 with a coefficient of determination R^2 = 0.9916. Table 3 shows the total phenolics content for the water extract, infus, and ethanolic extract. The ethanolic extract contained the highest amount of total phenolics, followed by infus and the water extract. Ethanol is a selective solvent and extracts more phenolic compounds compared to water [15].

Table 3. Total Phenolics Content

Samples	TPC (mg GAE/g extract)*
Water extract of *G. ulmifolia* leaves	27,503 ± 5,462[a]
Infus of *G. ulmifolia* leaves	40,772 ± 2,504[b]
Ethanolic extract of *G. ulmifolia* leaves	69,848 ± 1,871[c]

*TPC = total phenolics content. Data were presented as means ± SD (n = 3), different notation showed significant difference ($p<0.01$)

Total Flavonoids Content

The total flavonoids content was calculated from the quercetin standard curve using the correlation equation y = 0.0141x + 0.0966 with a coefficient of determination R^2 = 0.9978. Table 4 shows the total flavonoids content for the water extract, infus, and ethanolic extract. Similar to the total phenolics content, the ethanolic extract had the highest total flavonoids content, followed by infus and the water extract. Ethanol is also more selective in extracting flavonoids compared to water [15].

Table 4. Total Flavonoids Content

Samples	TFC (mg QE/g extract)*
Water extract of *G. ulmifolia* leaves	32,926 ± 0,477[c]
Infus of *G. ulmifolia* leaves	46,203 ± 2,449[b]
Ethanolic extract of *G. ulmifolia* leaves	118,854 ± 1,001[a]

*TFC = total flavonoids content. Data were presented as means ± SD (n = 3), different notation showed significant difference ($p<0.01$)

Correlation between Total Phenolics Content and Total Flavonoids Content on Antioxidant and Antiamylase Activity

Multiple regression analysis (Table 5) showed that total phenolics and flavonoids content did not concurrently affect the antioxidant (F-test was 9.183, less than F-table value of 10.295) nor the antiamylase activity (F-test was 0.137, less than F-table value of 10.295). Similarly, total phenolics, total flavonoids, and antioxidant activity did not affect the antiamylase activity (F-test was 2.123, less than F-table value of 7.519). The t-test of each dependent variable on each independent variable (Table 5) was less than t-table at a 99% confidence level, suggesting that they were not independently affecting or partially affecting the dependent variable.

The Pearson analysis results (Table 6) showed that total phenolics content was strongly correlated with antioxidant activity (Pearson correlation value of 0.868) but did not correlate with antiamylase activity (Pearson correlation value of -0.126). Total flavonoids content strongly correlated with antioxidant activity (Pearson correlation value of 0.864) but did not show correlation with antiamylase activity (Pearson correlation value of -0.145). Furthermore, antioxidant activity was uncorrelated with antiamylase activity (Pearson correlation value of 0.244).

Table 5. Multiple Regression Analysis Results

Dependent variable* / Independent variable	Antioxidant		Antiamylase		Antiamylase	
	F_{test}	F_{table}	F_{test}	F_{table}	F_{test}	F_{table}
Total phenolic content	9.183	10.295	0.137	10.295	2.123	7.591
Total flavonoids content						
Antioxidant						
	t_{test}	t_{table}	t_{test}	t_{table}	t_{test}	t_{table}
Total phenolic content	0.423	2.896	0.377	2.896	0.088	2.718
Total flavonoids content	0.097		-0.418		-0.658	
Antioxidant					2.423	

*Data was analyzed using p 0.01

Table 6. Pearson analysis results

Dependent variable / Independent variable	Antioxidant		Antiamylase	
	Pearson corr.	Sig.	Pearson corr.	Sig.
Total phenolic content	0.868*	0.002	-0.126	0.747
Total flavonoids content	0.864*	0.003	-0.145	0.709
Antioxidant			0.244	0.527

* Correlation is significant at the 0.01 level (2-tailed).

Based on the results, it is clear that infusion is the superior extraction method when looking to achieve the best antioxidant (Table 1) and antiamylase activities (Table 2). The lower the IC50, the higher the activity. Evidence supports that the main components of potential antioxidants are flavonoids and phenols [17]. However, this extraction method did not yield the highest total phenolics (Table 3) and flavonoid content (Table 4), which suggests that the antioxidant and antiamylase activities of the G. ulmifolia leaf infusion may be influenced by other bioactive compounds (Table 5). It has been stated that the presence of alkaloids and saponins in plants is particularly significant in the pharmaceutical field [18]. Alkaloids and saponins present in G. ulmifolia leaves could contribute to its antioxidant property. Some alkaloids, such as carbazomadurin A and B, cordifoline, carquinostatin A, oleracein A, B, and E, have proven scavenging activity against DPPH free radicals. The major factor influencing the antioxidant activity of alkaloids seems to be the number of aromatic hydroxyl groups [19]. The antioxidant activity may also be facilitated by saponins. There is a correlation between saponins and antioxidant activity, including the scavenging activity on DPPH free radicals [20].

The antiamylase activity of G. ulmifolia leaves infus is likely contributed by other metabolites besides phenolics and flavonoids. Alkaloids (such as broussonetine and radicamin) and triterpene saponins (such as arjunolic acid) are examples of non-phenolics and flavonoids secondary metabolites that can inhibit amylase enzymes [21]. According to the results of another study, ethanolic extracts with the highest concentration of total phenolic and flavonoid did not exhibit the best amylase inhibitor activity. Several active phytochemicals, including terpenes and flavonoids, among others, are thought to be responsible for the extract's potential anti-diabetic activity,

although this is not yet certain [22]. It has also been demonstrated that primary metabolites like gum tragacanth can inhibit amylase [23].

Contrarily, the ethanolic extract's total phenolics and flavonoids content was higher than that of the water extract and infus. Since phenolic compounds and flavonoid aglycones are semi-polar and dissolve in ethanol, the ethanolic extract, despite having the lowest yield, has the highest total phenolics and flavonoids content [24]. The yield from water extraction was higher than that from infusion. As previously stated, a water extract was made by heating to 100 ºC (boiling water), whereas an infusion was produced by heating to 90 ºC (in a water bath). These findings demonstrate that temperature impacts extraction results. An increase in temperature can improve extraction outcomes by increasing the solubility of secondary metabolites in dried leaf powder [15].

Considering its antioxidant and antiamylase activity, this study justifies the use of G. ulmifolia leaves in traditional medicine for diabetes management. This implies a direct use, as traditionally done in Indonesia, using the infusion method, not with other organic solvent [9]. Therefore, these leaves could serve as an alternative or complementary strategy for managing diabetes and could potentially be a raw material for developing anti-diabetic drugs in the future. Thus, further research is needed to determine which compounds in G. ulmifolia leaves infusion contribute to antioxidant and antiamylase activities.

CONCLUSION:

In conclusion, extraction methods impact the ability to scavenge free radicals, but not the ability to inhibit amylase. These methods also affect the total phenolic and flavonoid content. Infusion is the most effective method to obtain an extract with the best antioxidant and antiamylase activities, although it does not yield an extract high in total phenolic and flavonoid content. Nevertheless, further research is needed to determine which compounds in G. ulmifolia leaves infusion contribute to both antioxidant and antiamylase activities.

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CONFLICT OF INTEREST:

The authors hereby declare that regarding the publication of this paper there is no conflict of interests.

REFERENCES

1. Pereiraa GA., Araujoa NMP., Arrudaa HS., de Paulo Fariasa D., Gustavo Molinab G., Pastore GM. Phytochemicals and biological activities of mutamba (*Guazuma ulmifolia* Lam.): A review. *Food Research International.* 2019; 126(108713):1-19.
2. Mardisiswojo S. and Rajakmangunsudarso H. *Cabe Puyang Warisan Nenek Moyang*; Balai Pustaka: Jakarta 1985; 227.
3. Adnyana I. K., Yulinah E., Yuliet, and Kurniati, N. F. Antidiabetic activity of aqueous leaf extracts of *Guazuma ulmifolia* Lamk., ethanolic extracts of Curcuma xanthorrhiza and their combinations in alloxan-induced diabetic mice. *Research Journal of Medicinal Plant*. 2013; 7:158–164.
4. Pascoe-González, S., Ramos-Zavala, MG., Ahued, MAB., Hernández-González, SO., Cardona-Muñoz, EG., García-Benavides, L., and Grover-Páez, F. Administration of Herbarium Mixture (*Guazuma ulmifolia/Tecoma stans*) on Metabolic Profile in Type 2 Diabetes Mellitus Patients: A Randomized, Double-Blind, Placebo-Controlled Trial. *J Med Food.* 2021; 24(5):527–532.
5. Alonso-Castro, A. J. and Salazar-Olivo, L. A. The anti-diabetic properties of *Guazuma ulmifolia* Lam are mediated by the stimulation of glucose uptake in normal and diabetic adipocytes without inducing adipogenesis. *J Ethnopharmacol*. 2008; 118:252–256.
6. Kashtoh, H. and Baek, K. H. New Insights into the Latest Advancement in α-Amylase Inhibitors of Plant Origin with Anti-Diabetic Effects. *Plants*. 2023; 12(2944):1-28.
7. Qamar, A. Antidiabetic activity, polyphenols-based characterization and molecular interaction of extract of un-ripe pods of Vinca rosea cv. Pink. *Jordan j. pharm. Sci*. 2022; 15 (2):158-172.
8. Mahnashi, MH., Alqahtani, YS., Alyami, BA., Alqarni, AO., Alqahl, SA., Ullah, F., Sadiq, A., Zeb, A., Ghufran, M., Kuraev, A., Nawaz, A., and Ayaz, M. HPLC-DAD phenolics analysis, α-glucosidase, α-amylase inhibitory, molecular docking and nutritional profiles of *Persicaria hydropiper* L. *BMC Complement Med Ther.* 2022; 22(26):1-20.
9. Rafi, M., Meitary, N., Septaningsih, DA., and Bintang, M. Phytochemical Profile and Antioxidant Activity of *Guazuma ulmifolia* Leaves Extracts Using Different Solvent Extraction. *Indonesian Journal of Pharmacy Indonesian J Pharm*. 2020; 31(3):171-180.
10. Ministry of Health Republic of Indonesia. *Indonesian Pharmacopeia 4th Edition*. 1995.
11. Ministry of Health Republic of Indonesia. *Indonesian Herbal Pharmacopeia 2nd Edition*. 2017.
12. Gulcin, İ. and Alwasel, S. H. DPPH Radical Scavenging Assay. *Processes*. 2023; 11: 2248.
13. Thummajitsakul, S., Paensanit, P., Saeieo, T., Sirirat, J., and Silprasit, K. FTIR and multivariate analysis of total phenolic content, antioxidant and anti-amylase activities of extracts and milk of *Glycine max* L. and Phaseolus vulgaris L. *Electronic Journal of Biotechnology*. 2023; 64:69–75.
14. Purwaningsih, I., Fathiah, F., Amaliyah, N., and Kuswiyanto, K. The Phenolic, Flavonoid, and Anthocyanin Content from Methanol Extract of Senggani Fruit and Its Antioxidant Activity. *Indo. J. Chem. Res.* 2023; 10:195–202.
15. Lefebvre, T., Destandau, E., and Lesellier, E. Selective extraction of bioactive compounds from plants using recent extraction techniques: A review. *Journal of Chromatography A*. 2021: 1635.
16. Kumar, N. S. and Gurunani, S. G. *Guazuma ulmifolia* Lam: A review for future view. *Journal of Medicinal Plants Studies*. 2019; 7:205–210.

17. Naskar, A., Dasgupta, A. and Acharya, K. 2023. Antioxidant and Cytotoxic Activity of *Lentinus fasciatus*. *Jordan j. pharm. sci*. 2023; 16(1):72-81.
18. 18 Jemal, K., Sandeep, BV., and Pola, S. Phytochemical screening and *in vitro* antioxidant activity analysis of leaf and callus extracts of *Allophylus serratus (ROXB) KURZ. Jordan j. pharm. sci*. 2022; 15(1):51-69.
19. Macáková, K., Afonso, R., Saso, L. and Mladěnka, P. The influence of alkaloids on oxidative stress and related signaling pathways. *Free Radical Biology and Medicine*. 2019; 134:429–444.
20. Desta, KT., Yoon, H., Shin, MJ., Lee, S., Wang, X., Choi, YM., Jeon, Y., Ju, Y., and Yi, J. Assessment of Phenolic Content, Saponin Content, and Antioxidant Activities in Gray, Red, and White Adzuki Bean Germplasm: A Multivariate Analysis. *Korean J. Crop Sci*. 2023; 68:147–166.
21. Mogole, L., Omwoyo, W. and Mtunzi, F. Phytochemical screening, antioxidant activity and α-amylase inhibition study using different extracts of loquat (*Eriobotrya japonica*) leaves. *Heliyon*. 2020; 6.
22. Vadivelan, R., Gopala Krishnan, R. and Kannan, R. Antidiabetic potential of *Asparagus racemosus* Willd leaf extracts through inhibition of α-amylase and α-glucosidase. *J Tradit Complement Med*. 2019; 9:1–4.
23. Singh, P. and Gilhotra, R. M. Potent α-amylase inhibition activity of natural gums: An in-vitro anti-diabetic study. *International Journal of Pharmaceutical Research*. 2020; 12:196–200.
24. Che Sulaiman, IS., Basri, M., Masoumi, HRF., Chee, WJ., Ashari, SE. and Maznah Ismail, M. Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of *Clinacanthus nutans* Lindau leaves by response surface methodology. *Chem Cent J*. 2017; 11(54):1-11.

دراسة تأثير طرق الاستخلاص على النشاط البيولوجي ومضاد الأكسدة ومضاد الأميليز (والكيميائي) الفينول الكلي والفلافونويد *Guazuma ulmifolia* (لأوراق نبات)

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ملخص

تم تصميم هذه الدراسة لتقييم أنشطة مضادات الأكسدة ومضادات الأميليز، بالإضافة إلى إجمالي محتوى تم تصميم هذه الدراسة لتقييم أنشطة مضادات الأكسدة ومضادات الأميليز، بالإضافة إلى إجمالي محتوى الفينول والفلافونويد في مستخلصات أوراق *G. ulmifolia* المنتجة بطرق استخلاص مختلفة مستخدمة في إندونيسيا. تم اختبار نشاط مضادات الأكسدة باستخدام طريقة الاختزال 2,2-diphenyl-1-picrylhydrazyl (DPPH). تم اختبار نشاط مضاد الأميليز باستخدام اختبار قياس الألوان باستخدام حمض الساليسيليك 3,5-dinitro salicylic acid (DNSA) كركيزة. وفي الوقت نفسه، تم اختبار إجمالي نشاط مضادات الأكسدة تم قياس محتوى الفلافونويد باستخدام الطريقة اللونية. تم الحصول على أعلى عائد لاستخراج أوراق *G. ulmifolia* من مستخلص الماء (9.64%). أظهر التسريب أعلى نشاط مضاد للأكسدة ومضاد الأميليز (IC_{50} = 6.853 ± 0.504 ميكروغرام/مل و261.03±6.83 ميكروغرام/مل). وفي الوقت نفسه، تم العثور على أعلى محتوى إجمالي من الفينول والفلافونويد في مستخلص الإيثانول، حيث بلغ 69±1.871 ملجم من مستخلص GAE / جرام و854.118±1,001 ملجم من التيسير الكمي / جرام، على التوالي. تأثر نشاط مضادات الأكسدة بشكل كبير بالمحتوى الكلي للفينول والفلافونويد، لكن نشاط مضاد الأميليز لم يتأثر. في الختام، التسريب هو أفضل طريقة استخلاص للحصول على نشاط مضاد الأميليز العالي، على الرغم من أنه لا يوفر أعلى إنتاج وأعلى محتوى إجمالي من الفينول والفلافونويد. يجب إجراء المزيد من الأبحاث لمعرفة المركبات الموجودة في منقوع أوراق *G. ulmifolia* التي تساهم في نشاط مضادات الأكسدة ومضادات الأميليز.

الكلمات الدالة: *Guazuma ulmifolia*، طريقة الاستخلاص، مضاد الأكسدة، مضاد الأميليز، الفينول، الفلافونويد.

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Formulation and Evaluation of Herbal Emulsion-Based Gel Containing Combined Essential Oils from *Melaleuca alternifolia* and *Citrus hystrix*

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ABSTRACT

Aims: This study aimed to investigate the synergistic anti-inflammatory effects of combined essential oils from Melaleuca alternifolia and Citrus hystrix peel.

Methodology: An emulsion-based gel containing combined essential oils from Melaleuca alternifolia and Citrus hystrix peel was topically applied to the injected area of the carrageenan-induced hind paw edema mouse model. Diclofenac sodium was used as a positive control. The inhibition percentage of protein denaturation in all mice was assessed and calculated based on paw volume (Pv).

Results: The highest inhibition percentage, recorded at the third hour in the carrageenan-induced hind paw edema mouse model, was 44.76%, achieved by the mice treated with combined essential oils. This was followed by diclofenac-treated (37.14%), tea tree essential oil-treated (27.62%), and kaffir lime peel essential oil-treated mice (20.10%). A similar trend was observed after 24 hours of treatment, with the anti-inflammatory activity of combined essential oils-treated mice recorded at 46.74%, followed by diclofenac-treated (35.87%), tea tree essential oil-treated (24.97%) and kaffir lime peel essential oil-treated mice (11.97%).

Conclusion: These findings confirm the synergistic anti-inflammatory effects of the combined essential oils-incorporated emulsion-based gel on the carrageenan-induced hind paw edema mouse model.

Keywords: *Melaleuca alternifolia*, *Citrus hystrix*, essential oil, anti-inflammation, carrageenan-induced, edema.

INTRODUCTION

According to the World Health Organization (WHO), medicinal plants could become the best source to obtain a variety of medicine [1]. Approximately 80% of individuals from developed countries use traditional medicine, which contains compounds derived from medicinal plants [2]. Essential oils are aromatic oily liquids (also known as volatile oils) obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots) [3]. Of the estimated 3,000 known essential oils, some have been used for the treatment of various diseases while others have been utilized in food preservation or fragrance industries. Essential oils and their components are gaining increasing interest due to their ready availability, non-toxicity, and safety.

Melaleuca alternifolia (M. alternifolia) essential oil or tea tree oil (TTO) is a mixture of natural volatile constituents mainly extracted from the leaves of M. alternifolia, an evergreen shrub growing up to 6 m, predominantly found in Australia [4,5]. TTO is naturally present throughout the year in the leaf, but only found in the flower in June. TTO is recognized as a potential agent against microbial species and is now commonly used in pharmaceutical and cosmetic applications. TTO consists

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of many terpenes and their derivatives, of which terpinen-4-ol, α-pinene, linalool, and α-terpineol are considered the most important components having an antimicrobial effect [6, 7]. As revealed in prior studies, TTO exhibits a wide spectrum of antimicrobial activity against pathogenic microorganisms including Gram-positive and Gram-negative bacteria, yeasts, and fungi. Additionally, TTO has shown promise as a growth-inhibiting agent against multi-resistant microbes. Furthermore, TTO has been found effective in reducing inflammation, and is consequently widely incorporated as an active ingredient in numerous topical formulations for the treatment of various skin conditions such as wounds, acne, and contact dermatitis [8]. Given that high concentrations of TTO have been linked to irritation and side effects in humans, the combination of TTO with another essential oil such as Citrus limon leaves, Piper nigrum leaves [9], or Piper beetle leaves [10] is being researched. This can potentially lower the required dosage of TTO while also enhancing the therapeutic efficacy of blended essential oils.

Citrus hystrix (C. hystrix), or kaffir lime, is an evergreen shrub that grows up to 3 meters at a moderate rate. Its flowers are pollinated by insects and are self-fertile. Essential oil from the peel of C. hystrix, or kaffir lime peel essential oil (KPO), has been found to possess various significant biological activities and is touted for topical applications. Moreover, the essential oil derived from the peels and leaves is extensively used in the cosmetics and pharmaceutical industries, due to their significant pharmacological properties. These properties are primarily attributed to major phyto-constituents present in KPO, including β-pinene, α-pinene, D-limonene, and terpinen-4-ol [11, 12]. Additionally, KPO demonstrates inhibitory effects on human skin enzymes, including tyrosinase and hyaluronidase [13]. According to the Food and Agriculture Organization (FAO) statistics, roughly 60% of 998.7 thousand tons of citrus fruit waste is discarded per year in Vietnam [14]. Hence, using the waste peel from citrus fruits has gained considerable interest from researchers, due to its environmentally friendly nature, for developing C. hystrix-based healthcare products like soap [15] and mouthwash [16]. This study was primarily focused on investigating the synergistic anti-inflammatory efficacy of a gel incorporating a combination of tea tree and kaffir lime essential oils.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals were procured from the Pharmaceutical Chemistry Laboratory and Applied Biochemistry Laboratory of the Applied Biochemistry Department in International University Ho Chi Minh City, Vietnam. Carrageenan, triethanolamine (TEA), bovine serum albumin (BSA), Tween 80, and Carbomer 940 were bought from Sigma-Aldrich (USA). Phosphate buffered saline (PBS) buffer was prepared by dissolving a PBS tablet from Merck (Germany) in 1 liter of deionized water. Glycerol was a product of Merck (Germany), and Diclofenac sodium 75 mg/3mL was a product of Voltaren (Novartis, Switzerland). All chemicals and reagents were stored according to stringent regulations and freshly prepared with distilled water at desired concentrations for experimental purposes.

Extraction of Essential Oils

Fresh M. alternifolia leaves and C. hystrix peels were collected from a farm in Southern Vietnam, then thoroughly washed with running water to eliminate potential contaminants. The plant materials were air-dried and cut into small pieces prior to the extraction of the essential oil. These fresh plant materials were subjected to microwave-assisted extraction using a NEOS Milestone appliance for 90 minutes under the power of 900 W (yields an extract temperature of 100-110°C) [17, 18]. The resulting volatile oils were stored in amber vials and refrigerated at 4°C until analysis.

In vitro Anti-inflammation Assay

The anti-inflammatory properties of KPO and TTO were evaluated using a modified anti-protein denaturation

of the bovine serum albumin assay [19]. This assay was prepared by mixing 0.5 mL of test solution, comprised of 0.45 mL of BSA (5% w/v aqueous solution), and 0.05 mL of each essential oil at varying concentrations. Diclofenac sodium was chosen as a positive control, while de-ionized water was used as a negative control. All solutions were adjusted to a pH of 6.3 using 1N hydrochloric acid (109057, Merck, Germany). The samples were incubated at 37°C for 30 minutes, after which the temperature was increased to 57°C for another 3 minutes. Subsequently, the reaction mixture was allowed to cool at room temperature before adding 2.5 mL of PBS buffer. The absorbance was recorded at 660 nm using a spectrophotometer (Therma Scientific, USA) and a BioTek Synergy HT microplate reader. The percentage of inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Abs control solution-Abs test solution}}{\text{Abs control solution}}$$

The calibration curve was established using the regression equation y = bx + a. The anti-inflammatory activity of the essential oil was expressed as an IC50 value (µg/mL), which represented the concentration of essential oil causing 50% inhibition of protein denaturation. This was then compared with a sample containing the reference drug, diclofenac sodium 75 mg/mL.

Emulsion-based Gel Formulation

The emulsion-based gel, referred to as "emulgel," was formulated following the design protocol of Wulansari, Jufri, & Budianti, 2017 [20]. It was prepared in two phases using the basic ingredients as detailed in Table 1. The oily phase was prepared by dissolving Tween-80 in distilled water, followed by the addition of the essential oils (either KPO and TTO separately or in combination). Glycerol was added to this mixture and homogenized at 2,000 rpm for 15 minutes. In a separate container, Carbomer 940 was dispersed in distilled water before the addition of TEA to attain a pH in the range of 5.0-6.5. The preparation of the emulgel continued as the oily phase was combined with the aqueous phase under continuous agitation. This was completed by adding distilled water to make up 100 g of the mixture. The combination was homogenized using a homogenizer for 30 minutes until a smooth, clump-free gel with good integrity was obtained. The resultant gel formulations were transferred into sterile airtight containers, sealed, and stored in a cool area.

Table 1. Formulation of emulgel

	Ingredient	Amount (%)
Oily phase	Tween 80	30.00
	Glycerol	20.00
	Essential oil (individual or combined)	KPO: 3.501 g TTO: 2.717 g
Aqueous phase	Carbomer 940	1.00
	TEA	pH adjuster
	Distilled water	Qs. 100%

Evaluation of Emulgel Formulation

Physical Evaluation

Physical characteristics including color, odor and feeling [21] of application of prepared emulgel were noted.

Determination of pH

The pH values were determined using a digital pH meter [20]. This was done by dissolving 1 g of the emulgel sample in 100 mL of deionized water. Before the pH measurements, the pH meter was calibrated using standard

buffer solutions (pH 4, 7, and 10). Each preparation was measured in triplicate.

Homogeneity

The sample was subjected to visual observation by smearing it on microscope slides at three different temperatures (4, 25, and 37 °C) [22]. The presence of any clumps or disintegration would be considered a sign of heterogeneity [23, 24, 25].

Viscosity

The viscosity of the preparation was measured using a Brookfield viscometer Model RV-E with a spindle S64, at a rotation rate of 0.6 rpm at 4, 25, and 37°C [26].

Spreadability

Spreadability was determined using an apparatus [27] made up of a wooden block controlled by a pulley, based on the slip and drag characteristics of the gel. An excess of gel (about 2 g) was placed on a fixed slide. This gel sample was then sandwiched between this slide and another of the same dimensions attached to a hook. A 1-kg weight was placed on top of the two slides for 5 minutes to expel air and provide a uniform gel film between the slides. The excess gel was scrapped off from the edges. The top plate was then subjected to a pull of 80 g. Spreadability was calculated using the following formula:

$$S = M \times L / T$$

Where S = Spreadability; M = Weight in the pan (tied to the upper slide); L = Length moved by the glass slide; and T = Time (in seconds) taken to separate the upper slide from the ground slide.

Stability

The stability of the sample was evaluated by observing changes in appearance, homogeneity, pH, spreadability, and viscosity at 25°C for a duration of 2 months.

Animal Preparation for Testing

Albino Wistar mice of either sex, weighing 27 ± 2 g, were procured from the Pasteur Institute (Ho Chi Minh, Vietnam) and allowed to acclimate in experimental conditions for a week. All animals were approved and maintained according to the Animal Experimental Handbook at the Cellular Reprogramming Laboratory, International University, Vietnam National University of Ho Chi Minh City (http://crl.bio.hcmiu.edu.vn/about-us/facilities/) [28] and in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition) [29]. Mice were kept in a controlled environment with a light and dark cycle of 12 hours, provided a standard diet and water ad libitum. Animals were fasted for 24 hours preceding the assay.

Skin Irritation Assay

The intact skin of the prepared mice was used to create an animal model. Back hairs were removed three days before the experiment. Animals were treated with the prepared emulgel daily for a week before the erythema and edema on the treated skin were examined [27, 30]. The Primary Dermal Irritation Index (PII) was calculated based on the number of erythema scores recorded for each period of time in order to classify the degree of irritation [31]:

$$PII = \frac{[(\Sigma \text{ erythema scores at } 1/24/48/72h/\text{day } 7 + \Sigma \text{ edema scores at } 1/24/48/72h/\text{day } 7)]}{5 \times \text{number of mice}}$$

Induction of Hind Paw Edema by Carrageenan and Assessment of Anti-inflammatory Activity on Mice Model

Mice were randomly divided into six groups of eight (n = 8). Group I was the physiological group (normal mice). Group II was treated with 5 mg/kg body weight of diclofenac (positive control). Group III was treated with the emulgel incorporating combined essential oils. Group IV was treated with the KPO-incorporated emulgel, while group V was treated with the TTO-loaded emulgel. Group VI was the negative control group. The induction of hind paw edema in mice was conducted by injecting 50 µL of 1% w/v carrageenan in saline solution into the plantar side of the right hind paw of the mice, one hour before each experiment. A test sample, consisting of 0.2 g of the emulgel or diclofenac, was topically applied onto the injected area of the hind paw and gently rubbed in using a gloved finger. Paw volume (Pv) was measured using a

Plethysmometer (Ugo Basile, Italy) at 0, 3, and 24 hours after administration. The percentage of inhibition of protein denaturation was calculated based on Pv using the following formula:

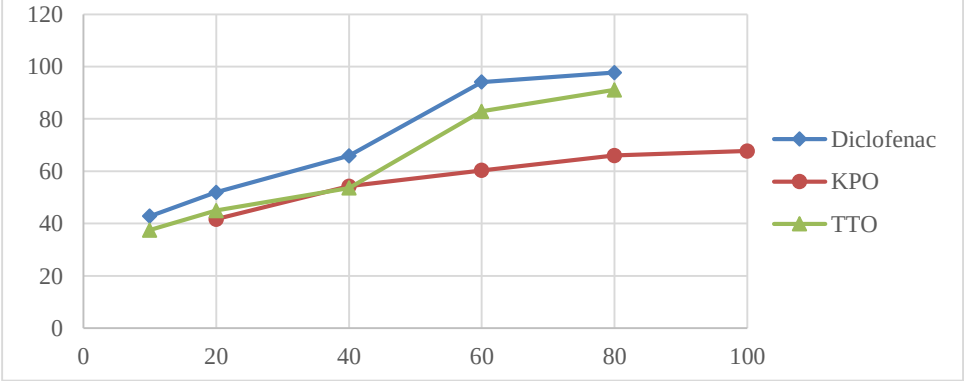
$$\% \, Inhibition = \frac{(Pv \; control - Pv \; test)}{Pv \; control} \times 100\%$$

Statistical Analysis

All experiments were conducted in triplicate. All values were reported as mean ± SD (standard deviation) and compared using an analysis of variance (ANOVA) for single-factor experiments. This was followed by Tukey's test to examine the significant difference between experimental data. Values with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In vitro Anti-inflammation



	Calibration curve	R^2	IC_{50} (µg/mL)
Diclofenac	Y = 0.8429 X + 35.092	0.9575	17.67 ± 0.023
KPO	Y = 0.3199 X + 38.797	0.9172	35.01 ± 0.024
TTO	Y = 0.8103 X + 27.977	0.9593	27.18 ± 0.028

Figure 1. In vitro anti-inflammatory activity of essential oil (KPO and TTO)

Figure 1 displays the concentration-dependent anti-inflammatory activity of essential oils against BSA denaturation. The reference drug, diclofenac sodium, exhibited potent anti-inflammatory activity, boasting the highest IC_{50} value of 17.67 ± 0.023 µg/mL. Meanwhile, TTO also demonstrated significant inhibition against protein denaturation with an IC50 value of 27.18 ± 0.028 µg/mL. This is in alignment with the value reported by John R.R et al. 2017 [32] which listed an IC50 value of 29.391 µg/mL. In contrast, KPO showed the least potential in preventing protein denaturation, with an IC50 value of 35.01 ± 0.024 µg/mL.

Physiochemical Evaluation of Emulgel

The emulgel was a shiny, jelly-white product that spread easily and had a firm, smooth texture. It possessed the specific aroma of eucalyptus and citrus peel (Figure 2). All emulgels produced a cooling sensation and adhered well to the skin, yet they could be easily removed with tap water. The pH of the prepared emulgel was slightly acidic (5.95 ± 0.055), which enhances permeability as it falls within the human skin pH range of 4.5-7.0 [33, 34]. All prepared emulgels exhibited good homogeneity upon visual and microscopic examination, and the average viscosity index was comparable to previous studies [35, 36]. Moreover, emulgels maintained their

homogeneity at different temperatures of 4, 25, and 40°C. Generally, all emulgels met the acceptable consistency conditions for application.

Figure 2. Emulgel state recorded at 25 oC

Stability Evaluation

The stability test confirmed that there were no statistically significant changes at room temperature (25oC) in pH ($p = 0.489$), viscosity ($p = 0.512$), and spreadability index ($p = 0.648$) (Table 2). The pH was observed to be under 6.0, which is considered acceptable to avoid the risk of irritation upon application to the skin. Thus, the formulation can be used in further effectiveness or clinical evaluations and scaled up for stability assessments in research and development scenarios.

Table 2. Stability of emulgel for a 2-month duration at 25°C

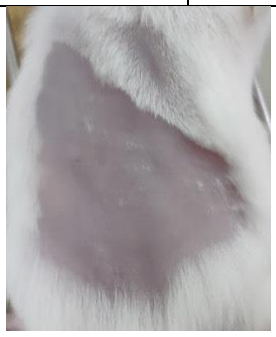
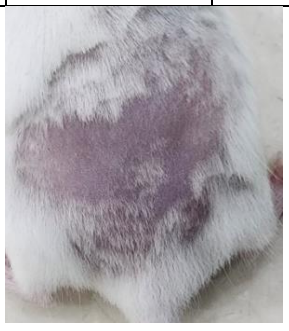
	Day 0	Day 15	Day 30	Day 45	Day 60
Organoleptic	Smooth, jelly white, well-homogenized, and greasy when applied.				
pH	5.95 ± 0.055	5.88 ± 0.087	5.83 ± 0.046	5.65 ± 0.562	5.95 ± 0.161
Viscosity (cPs)	4767.67 ± 88.94	4718.33 ± 22.74	4760.33 ± 40.67	4745.33 ± 22.37	4716.33 ± 42.64
Spreadability ($kg.m/s \cdot 10^{-4}$)	10.83 ± 0.06	10.83 ± 0.23	11.33 ± 0.15	10.93 ± 0.06	10.80 ± 0.36

Each value represents the mean ± SD (n = 3); $p > 0.05$.

Acute Skin Irritation

Skin irritation is characterized by the formation of erythema and edema, with the level of irritation calculated based on the PII [31], as presented in Table 3. As no erythema or edema was recorded on the mice throughout the test, the calculated PII was 0, indicating no irritation. The hair on the mice's backs was observed to grow normally after 7 days of the test.

Table 3. Skin irritation testing of emulgel

Irritation Score	24 h	48 h	72 h	7 days
Erythema	No case	No case	No case	No case
Edema	No case	No case	No case	No case
PII	0.0	0.0	0.0	0.0
				
Mouse's skin after shaved		Mouse's skin after 1-week assay		

Anti-inflammatory Activity of Emulgel on Carrageenan-induced Hind Paw Edema in Mice

The volume of the mice's hind paws was measured at 3 and 24 hours after carrageenan injection, and the level of inflammation reduction in all groups of mice was recorded, as summarized in Table 4. In general, there was a statistically significant difference in the inhibition percentage of protein denaturation among the tested groups after 3-hour treatment. However, there was no statistically significant difference within each of the six groups when comparing data obtained between the 3-hour and 24-hour treatments.

Table 4. Paw volume was documented in testing on carrageenan-induced hind paw edema in mice.

Group of mice (n = 8)	3 h		24 h	
	Pv (mL)	% Inhibition	Pv (mL)	% Inhibition
I	1.05 ± 0.05	0.00	0.92 ± 0.04	0.00
II	0.66 ± 0.05	37.14	0.59 ± 0.05	35.87
II	0.58 ± 0.05	44.76	0.49 ± 0.03	46.74
IV	0.84 ± 0.01	20.10	0.81 ± 0.05	11.97
V	0.76 ± 0.03	27.62	0.69 ± 0.03	24.97
VI	0.93 ± 0.06	11.43	0.88 ± 0.05	4.35

Each value represents the mean ± SD (n = 8); *$p < 0.05$ compared to control (I and VI)*

The emulgel loaded with a combination of essential oils significantly reduced the level of inflammation in a carrageenan-induced hind paw mouse model, compared to both the physiological control group and the negative control group. The combined essential oil emulgel demonstrated anti-inflammatory effects similar to those of diclofenac sodium. Conversely, the emulgel base alone was incapable of reducing inflammation compared to the physiological control. Both individual essential oils, TTO and KPO, displayed substantial inflammation reduction when compared to the physiological and negative control groups. However, samples that contained the combined essential oils demonstrated significantly higher anti-inflammatory activity in the carrageenan-induced hind paw edema mouse model compared to the samples loaded with a single essential oil. This effectively showcases the synergistic anti-inflammatory properties of the combined essential oil-loaded emulgel. After the third hour, the largest recorded percentage of inflammation inhibition was 44.76% yield by the group treated with the combined essential oils. This was followed in succession by the diclofenac-treated group (37.14%), TTO-treated group (27.62%), and KPO-treated group (20.10%). A similar trend was also observed after a 24-hour treatment period, with the anti-inflammatory activity of the combined essential oils-treated group recorded at 46.74%, followed by the diclofenac-treated group (35.87%), TTO-treated group (24.97%), and KPO-treated group (11.97%).

The data obtained from the current research demonstrated that the combination of TTO and KPO exhibited more pronounced anti-inflammatory effects on the carrageenan-induced hind paw edema mouse model than did a single essential oil. It is noteworthy that carrageenan-induced hind paw edema is a conventional in vivo model for acute inflammation [37]. This model is typically used to investigate new anti-inflammatory agents and drugs derived from natural sources. The inflammation induced by carrageenan is usually characterized by the release of inflammatory and pro-inflammatory mediators, including prostaglandins, cytokines, histamines, and bradykinins. Inflammation also involves the overproduction of free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) and may activate certain enzymes like cyclooxygenase (COX). According to

previous studies, the anti-inflammatory efficacy of essential oils can be largely attributed to the main components present in both KPO and TTO, including terpinen-4-ol, γ-terpinene, α-terpinene, p-cymene, α-terpineol, limonene, eucalyptol, citronellal, β-pinene, and sabinene. These phyto-components have been extensively studied using modern techniques and their mechanisms of action have been partially elucidated, playing a key role in interaction with components resulting from inflammation induced by carrageenan. Our findings contribute to the scientific understanding of herbal medicine and highlight the considerable potential of the synergistic anti-inflammatory effectiveness of combined essential oils from KPO and TTO. This combination could be potentially utilized in developing plant-based therapeutic agents for the topical treatment of various skin conditions, particularly those related to the immune system, such as atopic dermatitis.

CONCLUSION

In conclusion, this research demonstrates the synergistic anti-inflammatory efficacy of combined KPO and TTO-incorporated emulgel in a carrageenan-induced hind paw edema mouse model. We hope these findings will encourage further studies to elucidate the mechanisms behind the potential synergistic interaction between the natural intrinsic components of KPO and TTO. The ultimate goal is to exploit the potential of these valuable essential oils for topical treatment against skin conditions.

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Conflicts of Interest

We declare that there is no conflict of interest.

REFERENCES

1. Manandhar S., Luitel S., Dahal RK. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. *J Trop Med*. 2019; 2019:1-5. doi:10.1155/2019/1895340
2. Nascimento GGF., Locatelli J., Freitas PC., Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz J Microbiol*. 2000; 31(4). doi:10.1590/S1517-83822000000400003
3. Solórzano-Santos F., Miranda-Novales MG. Essential oils from aromatic herbs as antimicrobial agents. *Curr Opin Biotechnol*. 2012; 23(2):136-141. doi:10.1016/j.copbio.2011.08.005
4. Carson CF. and Riley TV. Antimicrobial activity of the essential oil of Melaleuca alternifolia. *Lett Appl Microbiol*. 1993; 16(2):49-55. doi:10.1111/j.1472-765X.1993.tb00340.x
5. Carson CF., Riley TV., Cookson BD. Efficacy and safety of tea tree oil as a topical antimicrobial agent. *J Hosp Infect*. 1998; 40(3):175-178. doi:10.1016/S0195-6701(98)90135-9
6. Carson CF. and Riley TV. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. *J Appl Bacteriol*. 1995; 78(3):264-269. doi:10.1111/j.1365-2672.1995.tb05025.x
7. Raman A., Weir U., Bloomfield SF. Antimicrobial effects of tea-tree oil and its major components on Staphylococcus aureus, Staph. epidermidis and Propionibacterium acnes. *Lett Appl Microbiol*. 1995; 21(4):242-245.

doi:10.1111/j.1472-765X.1995.tb01051.x

8. Hammer KA., Carson CF., Riley TV., Nielsen JB. A review of the toxicity of Melaleuca alternifolia (tea tree) oil. *Food Chem Toxicol*. 2006; 44(5):616-625. doi:10.1016/j.fct.2005.09.001
9. Nikolić MM., Jovanović KK., Marković TL., et al. Antimicrobial synergism and cytotoxic properties of *Citrus limon* L., *Piper nigrum* L. and *Melaleuca alternifolia* (Maiden and Betche) Cheel essential oils. *J Pharm Pharmacol*. 2017; 69(11):1606-1614. doi:10.1111/jphp.12792
10. Son L. Hoang, Nhi H.M. Nguyen. Antibacterial Synergism of Blended Essential Oils from Piper betle L. and Melaleuca alternifolia. *South Asian Research Journal of Natural Products*. 2023; 6(2):88-100.
11. Hien TT., Quyen NTC., Minh PTH., Le XT. Determine the components of Kaffir Lime Oil (Citrus Hystrix DC.) in the Microwave-assisted Extraction Process. *IOP Conf Ser Mater Sci Eng*. 2020; 991(1):012018. doi:10.1088/1757-899X/991/1/012018
12. Tsai ML., Lin CC., Lin WC., Yang CH. Antimicrobial, Antioxidant, and Anti-Inflammatory Activities of Essential Oils from Five Selected Herbs. *Biosci Biotechnol Biochem*. 2011; 75(10):1977-1983. doi:10.1271/bbb.110377
13. Hongratanaworakit T., Tapaneeyasin P., Nuamlert J., Chansiri A., Hongrattanavorakit N. Development of skin whitening preparations from kaffir lime oil (Citrus hystrix). *Planta Med*. 2006; 72(11):s-2006-950008. doi:10.1055/s-2006-950008
14. FAO report. *Citrus Fruit Statistics 2015*. Food and Agriculture Organization of the United Nations (FAO); 2016.
15. Dang HG., Nguyen THT., Le NiTA., et al. Distillation of kaffir lime (Citrus hystrix DC.) essential oil and making of antibacterial liquid soap. *Can Tho Univ J Sci*. 2022; 58(2):1-10. doi:10.22144/ctu.jvn.2022.031
16. Buakaew W., Sranujit RP., Noysang C., et al. Evaluation of Mouthwash Containing Citrus hystrix DC., Moringa oleifera Lam. and Azadirachta indica A. Juss. Leaf Extracts on Dental Plaque and Gingivitis. *Plants*. 2021; 10(6):1153. doi:10.3390/plants10061153
17. Quyen NTC., Ngan TTK., Phong HX., Hien TT., Dung LT. Extraction of Kaffir Lime (Citrus hystrix DC.) essential oil by steam distillation and evaluation of chemical constituents. *IOP Conf Ser Mater Sci Eng*. 2020; 991(1):012015. doi:10.1088/1757-899X/991/1/012015
18. Hoa DM., Chi NTD., Duy VH. Study on extraction, chemical component investigation and initial application of Melaleuca alternifolia (Tea tree) oil in mouthwash production. *Can Tho Univ J Sci*. 2016; 45:90. doi:10.22144/ctu.jvn.2016.515
19. Murugan R. and Parimelazhagan T. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from Osbeckia parvifolia Arn. – An in vitro approach. *J King Saud Univ - Sci*. 2014; 26(4):267-275. doi:10.1016/j.jksus.2013.09.006
20. Wulansari A., Jufri M., Budianti A. Studies on the formulation, physical stability, and in vitro antibacterial activity of tea tree oil (Melaleuca alternifolia) nanoemulsion gel. *Int J Appl Pharm*. 2017; 9:135. doi:10.22159/ijap.2017.v9s1.73_80
21. Abbadi, M. A., Darwish, L. H., Abu-Hijleh, S. Y. Formulation and evaluation of a novel topical gel for the treatment of acne vulgaris. *Jordan j. pharm. sci*. 2011; 1(1):18-27.
22. Khan BA., Ali A., Hosny KM., et al. Carbopol emulgel loaded with ebastine for urticaria: development, characterization, *in vitro* and *in vivo* evaluation. *Drug Deliv*. 2022; 29(1):52-61. doi:10.1080/10717544.2021.2015483
23. Linsinger TPJ., Pauwels J., Van Der Veen AMH., Schimmel H, Lamberty A. Homogeneity and stability of reference materials. *Accreditation Qual Assur*. 2001; 6(1):20-25. doi:10.1007/s007690000261

24. Guidance for characterization and assessment of homogeneity and stability.
25. General requirements for the competence of reference material producers.
26. Khan BA., Ahmad S., Khan MK., et al. Fabrication and Characterizations of Pharmaceutical Emulgel Co-Loaded with Naproxen-Eugenol for Improved Analgesic and Anti-Inflammatory Effects. *Gels*. 2022; 8(10):608. doi:10.3390/gels8100608
27. Mahendra A. Giri and Rasika D. Bhalke. Formulation and evaluation of Topical Anti-inflammatory herbal gel. *Asian J Pharm Clin Res*. Published online May 28, 2019: 252-255. doi:10.22159/ajpcr.2019.v12i7.33859
28. International University Doc. Animal Experimental Handbook at Cellular Reprogramming Laboratory. Published online 2013. http://crl.bio.hcmiu.edu.vn/about-us/facilities/
29. *Guide for the Care and Use of Laboratory Animals*. Vol 8th edition. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. National Academies Press (US); 2011.
30. Asad MI., Khan D., Rehman AU., Elaissari A., Ahmed N. Development and In Vitro/In Vivo Evaluation of pH-Sensitive Polymeric Nanoparticles Loaded Hydrogel for the Management of Psoriasis. *Nanomaterials*. 2021; 11(12):3433. doi:10.3390/nano11123433
31. OECD. *Test No. 404: Acute Dermal Irritation/Corrosion*. OECD; 2002. doi:10.1787/9789264070622-en
32. John Rozar Raj, Geetha. Anti-inflammatory effect of teatree oil. *Int J Curr Res*. 2017; 9(4):49171-49172.
33. Worth AP. and Cronin MTD. The use of pH measurements to predict the potential of chemicals to cause acute dermal and ocular toxicity. *Toxicology*. 2001; 169(2):119-131. doi:10.1016/S0300-483X(01)00494-2
34. Abbadi M.A., Al-Omari M.M., Abu-Hijleh S.Y., Al-Dabbagh M.A. Development and evaluation of a topical gel containing a combination of retinol and benzoyl peroxide for the treatment of acne vulgaris. *Jordan j. pharm. sci*. 2015; 5(1):56-65.
35. Rao M, Sukre G, Aghav S, Kumar M. Optimization of Metronidazole Emulgel. *J Pharm*. 2013; 2013:1-9. doi:10.1155/2013/501082
36. Kalayi M., Yeğen G., Okur NÜ., Aksu B. Evaluation of emulgel formulations contain diclofenac sodium via quality by design approach. *J Res Pharm*. 2022; 26(3):460-468. doi:10.29228/jrp.143
37. Posadas I., Bucci M., Roviezzo F., et al. Expression of Concern: Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. *Br J Pharmacol*. 2004; 142(2):331-338. doi:10.1038/sj.bjp.0705650
38. Ghorbanzadeh B., Mansouri M., Hemmati A., Naghizadeh B., Mard S., Rezaie A. A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. *Indian J Pharmacol*. 2015; 47(3):292. doi:10.4103/0253-7613.157127
39. Grazul M., Kwiatkowski P., Hartman K., Kilanowicz A., Sienkiewicz M. How to Naturally Support the Immune System in Inflammation—Essential Oils as Immune Boosters. *Biomedicines*. 2023; 11(9):2381. doi:10.3390/biomedicines11092381

صياغة وتقييم جل مستحلب عشبي يحتوي على زيوت عطرية مجمعة من نبات *Citrus hystrix* و*Melaleuca Alternifolia*

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ملخص

الأهداف :تهدف هذه الدراسة إلى دراسة التأثيرات التآزرية المضادة للالتهابات للزيوت العطرية المركبة من *Melaleuca Alternifolia* وقشر الحمضيات.

المنهجية :تم تطبيق هلام ذو أساس مستحلب يحتوي على زيوت أساسية مجمعة من Melaleucaالبديل وقشر الحمضيات موضعياً على المنطقة المحقونة من نموذج الفئران الوذمة الخلفية التي يسببها الكاراجينان .تم استخدام ديكلوفيناك الصوديوم كعنصر تحكم إيجابي .تم إخضاع جميع الفئران لتقييم نسبة تثبيط تمسخ البروتين والتي تم حسابها على أساس حجم المخلب .(Pv)

النتائج :تم تسجيل التأثيرات المضادة للالتهابات على وذمة المخلب الخلفي الناجمة عن الكاراجينان في الفئران في الساعة الثالثة مع أعلى نسبة تثبيط قدرها 44.76٪ في الفئران المعالجة بالزيوت العطرية مجتمعة تليها الفئران المعالجة بالديكلوفيناك 37.14)٪ (وشجرة الشاي .زيت أساسي معالج (27.62%)، وقشر الليمون الكافيري معالج بالزيت الأساسي .(20.10%) وبالمثل، لوحظ نفس الاتجاه أيضًا بعد العلاج لمدة 24ساعة مع النشاط المضاد للالتهابات للفئران المعالجة بالزيوت العطرية المجمعة المسجلة بنسبة 46.74%، يليها الفئران المعالجة بالديكلوفيناك (35.87%)، والفئران المعالجة بالزيوت العطرية لشجرة الشاي .(24.97%)وقشر الليمون الكافير المعالج بالزيت العطري .(11.97%)

الاستنتاج :أكدت هذه النتائج التأثيرات التآزرية المضادة للالتهابات للجيل القائم على مستحلب الزيوت العطرية المدمجة على نموذج الفئران الوذمة الخلفية التي يسببها الكاراجينان.

الكلمات الدالة: شجرة الشاي، الحمضيات، الزيوت العطرية، مضاد للالتهابات، الكاراجينان، الوذمة.

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Knowledge and Consumption Practice of Energy Drinks among Medical University Students in Mosul, Iraq

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ABSTRACT

Objective: Energy drinks are gaining popularity, and their consumption is associated with adverse effects. This study aims to explore the level of knowledge regarding energy drinks among medical students and the practices of those students who consume these products.

Methods: A cross-sectional, questionnaire-based study design was adopted for this work. The survey was distributed among students of medical colleges at the University of Mosul to assess their knowledge and practices regarding energy drinks.

Results: A total of 1298 students participated in the study, with 60% being females. Most of the students (89%) knew what energy drinks are, but only 42% knew their ingredients. Almost all the students (95%) were aware that energy drinks have adverse effects, but only a few knew about any beneficial effects these products might have. Only 30% of the participants admitted to consuming energy drinks, with more than half of those students drinking less than five cans monthly. Sugar-containing products were more favored than sugar-free ones, and Tiger™ was the most preferred brand of energy drinks among students. Forty-one percent of the energy drink-consuming students reported experiencing side effects. The knowledge of the side effects of energy drinks was the most commonly reported reason for not consuming these beverages. Older male students in their last two years of study and those living within the city were found to be more knowledgeable about energy drinks.

Conclusions: The level of knowledge regarding energy drinks was low, which should be a cause for concern, especially given the increasing popularity of energy drinks and the marketing campaigns targeting youth.

Keywords: Energy drinks, Tiger™, Knowledge, EDs consumption, Practice.

INTRODUCTION

"Energy drinks" (EDs) is the term given to beverages that are sold with the claim of boosting consumers' "energy" through their containment of caffeine in conjunction with a variety of other ingredients [1]. Despite the fact that the term "energy drinks" is not officially recognized by the American Food and Drug Administration (FDA) or the United States Department of Agriculture (USDA), these beverages represent a multibillion-dollar market and have gained increasing popularity in recent years, thus making them the fastest-growing sector in beverage sales [1-3]. A wide variety of brands and flavors of EDs are available in the market [4], all reputedly able to increase consumer energy through their content of stimulants and energy enhancers such as caffeine, guarana (a plant rich in caffeine), taurine, vitamins, sugar, ginseng, gingko biloba, among others [5].

Energy drinks are specifically marketed towards certain demographic segments such as truck drivers, university students, athletes, and other sectors of society that require an "energy boost" [6]. Some of the benefits attributed to

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consumption of EDs in these populations include improved mental and physical capabilities, increased athletic endurance and energy, enhanced learning ability, ability to maintain wakefulness for longer periods, faster reaction times, improved emotional health, and better driving skills [7]. However, the use of EDs also carries associated risks. Documented adverse effects of EDs include impacts on the cardiovascular system like palpitations and increased blood pressure [8], effects on the central nervous system such as restlessness, sleeping disturbances, and acute psychosis [5,9-11], the renal system (e.g., metabolic acidosis) [11,12], the endocrine system (e.g., obesity, hyperinsulinemia, decreased insulin sensitivity) [5,13,14], along with gastrointestinal upset, peptic ulcer [15,16], and increased dental erosion [17].

Despite the harmful effects of EDs, many individuals consume them excessively, leading to suggestions that warning labels should be placed on packaging [10, 18]. This is particularly common among university students, who often ingest high quantities of EDs to manage the stress associated with studying, assignments, examinations, and extended periods of separation from their families. Furthermore, many of these students are uninformed about the detrimental consequences these beverages can provoke—instead, they remain captivated by their stimulating effects [18-22].

Aligning with logical expectations, pursuing a degree in a medicine-related field should theoretically reduce the consumption of EDs since such students would learn about these beverages' harmful impacts—a proposition supported by existing literature [23]. Accordingly, this study strives to evaluate the level of knowledge regarding EDs among medical university students and to understand the practices these students adopt concerning these beverages in the city of Mosul, Iraq.

METHODS

A survey was conducted among medical college students at the University of Mosul to assess the knowledge and consumption practices related to EDs. Google Forms was utilized to create and disseminate the questionnaire. The survey was composed of three sections: the first comprised seven questions that collected sociodemographic data (age, gender, year of study, residence, sleep duration, sleep regularity, and students' weekly income).

The second part assessed the participants' knowledge about EDs, their constituents, and their effects on the body, using 18 questions. Participant knowledge was assessed by assigning a score of "1" for each correct answer and "0" for each incorrect answer, yielding a potential knowledge score range of 0 to 18. The median-split method [24] was employed to categorize students based on their scores into groups with either adequate or inadequate knowledge. The median was identified as 8, and thus, students with a knowledge score less than 8 were deemed to have inadequate knowledge, while those with a score of 8 or higher were categorized as having adequate knowledge.

The third section of the questionnaire comprised 11 practice questions, addressing aspects such as ED consumption, frequency and timing of ED consumption, preferred drinks and ED brands, reasons for selecting a specific brand, whether they experienced side effects (SE), the types of SE experienced, and the reasons for consuming EDs. Finally, a question asked participants who refrained from drinking EDs about their motivations for non-consumption.

The survey link was randomly distributed to students from October 13th to 20th, 2022, by sharing the link with different class representatives. These representatives then forwarded the survey link to their classmates via various social media groups, in which most students were members. Eligibility for this study was restricted to students in pharmacy, dentistry, or medical schools, excluding students from other schools and colleges within the university. Based on data procured from the Registration Offices at the Colleges of Pharmacy, Dentistry, and Medicine, the approximate total number of undergraduate students during data collection was 6,000. The Raosoft sample size calculator (http://www.raosoft.com/samplesize.html) was utilized to determine the sample size, identifying a minimum of 362 students as the desired size to achieve a 95% confidence level.

The survey was conducted in Arabic to encourage student participation and to provide ease in survey completion. The reliability of the survey items was tested through the calculation of the Cronbach's alpha value. The survey's content validity was verified using the opinions of three experts.

The study's aim was detailed in the first page of the Google Form. It was clearly indicated that participation was voluntary, with a mandatory tick box present on the first page to record students' informed consent to participate and complete the survey. Furthermore, prior to the beginning of data collection, the study received ethical approval from the Scientific Committee at the Department of Clinical Pharmacy, College of Pharmacy, University of Mosul.

Statistical analysis, which includes both descriptive and inferential statistics, was performed using SPSS version 28 software. A statistical significance level of P-value ≤ 0.05 was applied to all results.

RESULTS

A total of 1,298 medical students completed the questionnaire, constituting the final study sample. The Cronbach's alpha value was 0.769, indicating acceptable internal consistency. In terms of validity, the experts determined that the survey questions sufficiently reflected the topics needed to fulfill the study's objectives. The majority of the students (84.6%) were aged between 21 and 23 years old. Approximately 60% of the participants were female, and the percentages of students studying in the second, third, and fourth classes were 20%, 22%, and 21%, respectively. About 80% of the participants resided in urban areas, and approximately 57.9% reported irregular sleep patterns. The regular sleep duration for 64.8% of the students was between 6 and 8 hours. The weekly allowance from their families was less than $20 for approximately two-thirds of the students (60.9%). Table 1 provides a summary of the students' socio-demographic characteristics.

Table 1: Demographic characteristics of the students

Variables (N= 1298)	Frequency (%)
Age	
18 – 20 years	126 (9.7)
21 – 23 years	1098 (84.6)
> 23 years	74 (5.7)
Gender	
Male	516 (39.8)
Female	782 (60.2)
Year of study	
1st year	221 (17.0)
2nd year	262 (20.2)
3rd year	289 (22.3)
4th year	277 (21.3)
5th year	213 (16.4)
6th year	36 (2.8)
Residence	
Urban	1074 (82.7)
Rural	224 (17.3)
Sleeping regularly	
Yes	547 (42.1)
No	751 (57.9)
Sleeping hours	
< 6 hours	0 (0)
6 – 8 hours	841 (64.8)
> 8 hours	457 (35.2)
Weekly income	
< 20 $	791 (60.9)
20 – 35 $	382 (29.4)
> 35 $	125 (9.6)

Upon questioning students about the constituents of EDs, a mere 6.7% identified ginkgo biloba as an ingredient, while 18.2% and 19.0% mentioned taurine and vitamins, respectively. The majority of respondents (95.4%) were aware that EDs could have various negative health impacts and understood that EDs can affect both blood sugar levels (90.5%) and heart rate (89.7%). The response percentages to other knowledge-based questions, along with their correct answers, are presented in Table 2.

Table 2: Responses of the students to knowledge questions

	Knowledge Questions (N= 1298)	Answers	
		Yes n (%)	No n (%)
Q1	Do you know what energy drinks are?	1154 (88.9) *	144 (11.1)
Q2	Do you know what the ingredients of EDs are?	541 (41.7) *	757 (58.3)
Q3	EDs contain caffeine	798 (61.5) *	500 (38.3)
Q4	EDs contain taurine	236 (18.2) *	1062 (81.8)
Q5	EDs contain sugars	747 (57.6) *	551 (42.4)
Q6	EDs contain ginkgo biloba	87 (6.7) *	1211 (93.3)
Q7	EDs contain vitamins	247 (19.0) *	1051 (81.0)
Q8	EDs enhance physical performance	494 (38.1) *	804 (61.9)
Q9	EDs improve concentration/ability to study	415 (32.0) *	883 (68.0)
Q10	EDs help to stay awake	770 (59.3) *	528 (40.7)
Q11	EDs cause weight gain	316 (24.3) *	982 (75.7)
Q12	EDs enhance metabolism	84 (6.5) *	1214 (93.5)
Q13	EDs improve mood	201 (15.5)	1097 (84.5) *
Q14	EDs have other effects	233 (18.0) *	1065 (82.0)
Q15	Do EDs have negative effects?	1238 (95.4) *	60 (4.6)
Q16	EDs raise blood pressure	960 (74.0) *	338 (26.0)
Q17	EDs affect blood sugar	1175 (90.5) *	123 (9.5)
Q18	EDs affect heart rate	1164 (89.7) *	134 (10.3)

* Correct answer

Thirty percent of the medical students reported consuming EDs (Figure 1A). When queried about their reasons for consuming these beverages, the most common response was for study purposes (175 students), followed by no specific reason (169 students). Meanwhile, 128 students reported drinking EDs to stay awake longer. Drinking EDs for sports, driving long distances, and friends' encouragement were less commonly reported reasons with 57, 42, and 31 responses respectively (Figure 1B). Table 3 features the students' responses to practice questions. A third of the respondents (35.2%) claimed to consume EDs one to three times a week, more than half (58.6%) reported drinking less than five cans a month, and 47.7% stated that they would consume EDs at any time during the day.

Table 3: Responses of the students to the practice questions

Variables (N=384)	Frequency (%)
Frequency of drinking EDs	
Daily	59 (15.4)
More than once weekly	108 (28.1)
Once weekly	82 (21.4)
1 – 3 times monthly	135 (35.2)
Number of cans drunk monthly	
Less than 5 cans	225 (58.6)
5 – 10 cans	87 (22.7)
10 – 20 cans	44 (11.5)
More than 20 cans	28 (7.3)
Time to drink EDs	
At morning	51 (13.3)
With meals	12 (3.1)
At night	138 (35.9)
Anytime	183 (47.7)

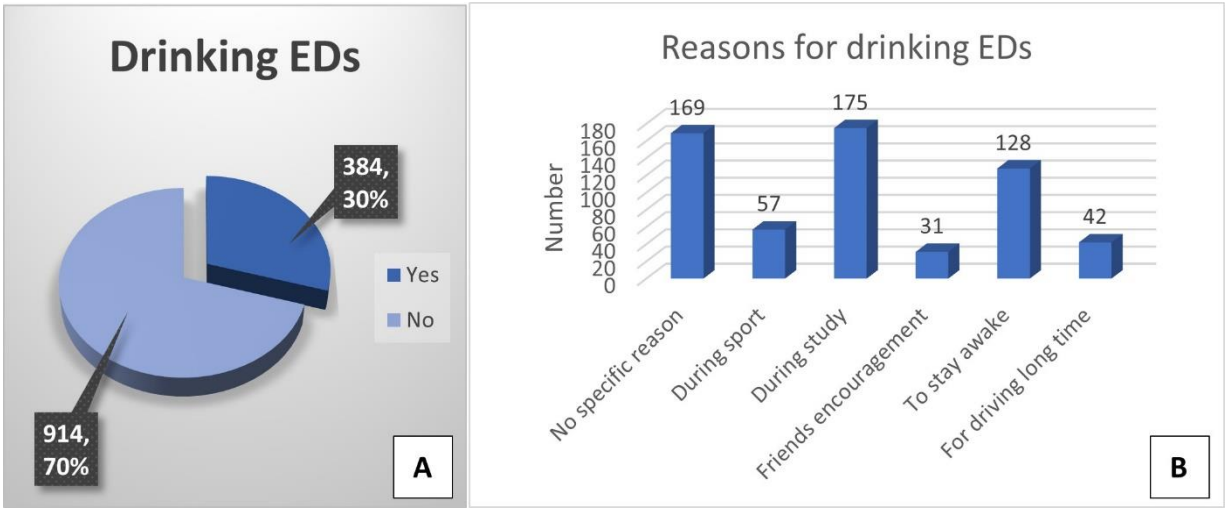

Figure 1: A: Frequency of students who drink EDs
B: Reasons behind drinking EDs (more than one answer per student)

When inquired about the students' preferred types of drinks, 77% indicated a preference for beverages containing sugar (Figure 2A). With regard to their preferred brand of EDs, Tiger™ was the most popular, selected by 48.7% of respondents, followed by Red Bull (24.0%), Monster Energy (15.4%), and Smart (7.8%) (Figure 2B). The primary reasons for choosing a specific brand of EDs were taste and the drink's effect (Figure 2C).

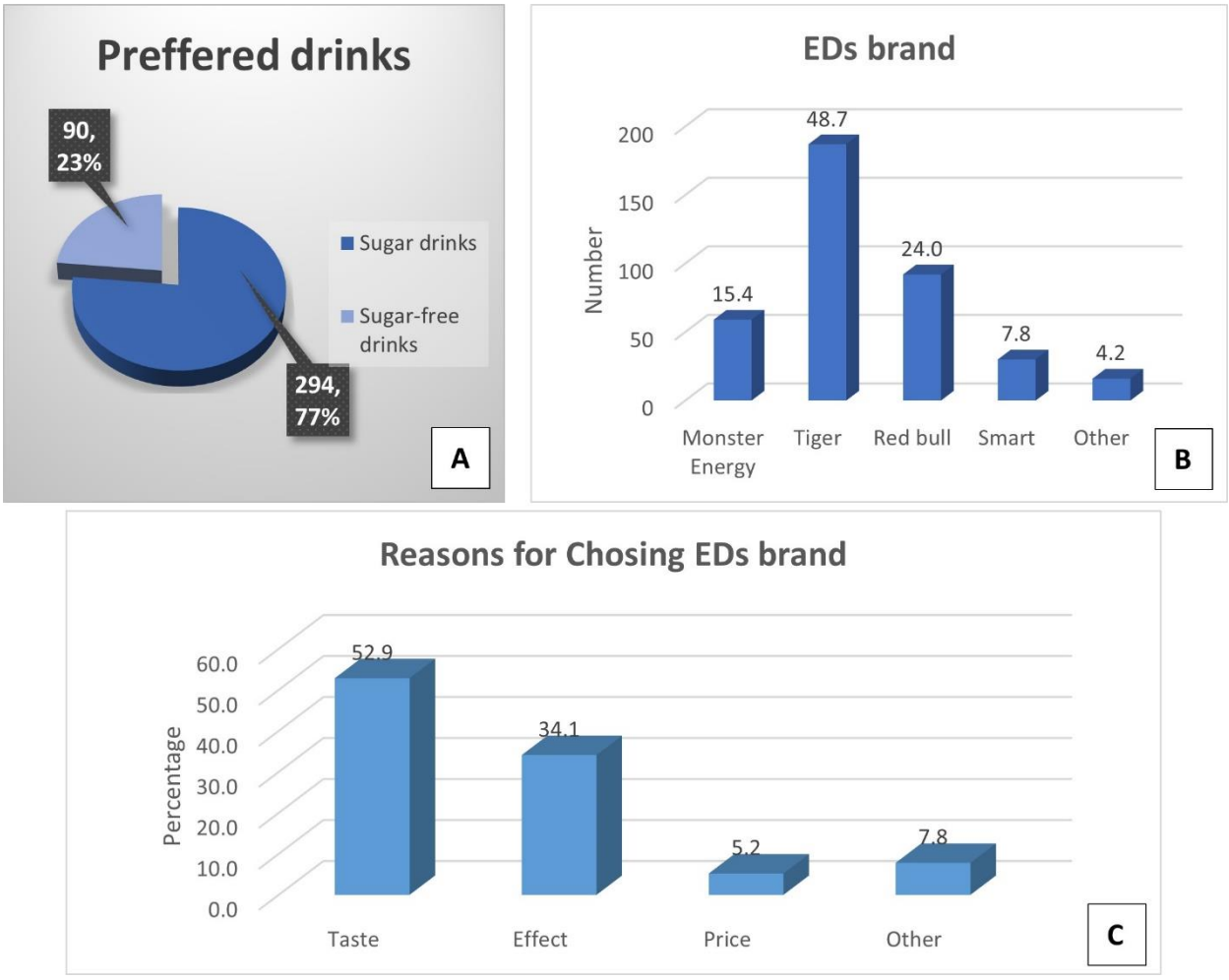

Figure 2: A: Preferred EDs in terms of sugar content
B: Preferred EDs brand
C: Reasons for preferring ED brands

Approximately 71% of students reported that EDs met their expectations. As shown in Figure 3A, 226 respondents (59%) had experienced some side effects following consumption of EDs. The most frequently reported effects were insomnia and palpitations, followed by fatigue, nervousness, and anxiety (Figure 3B).

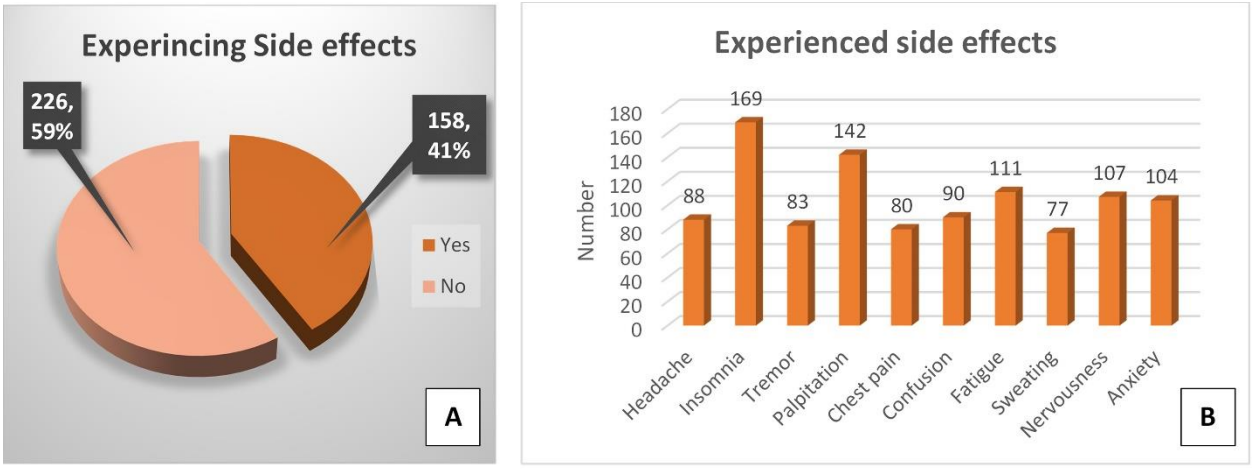

Figure 3: A: Experiencing side effects from EDs
B: Frequency of different side effects experienced by students (more than one answer per student)

Upon questioning the medical students who refrained from consuming EDs about their reasoning, the majority indicated that knowledge of EDs' side effects or a general dislike for EDs were the primary reasons (Figure 4).

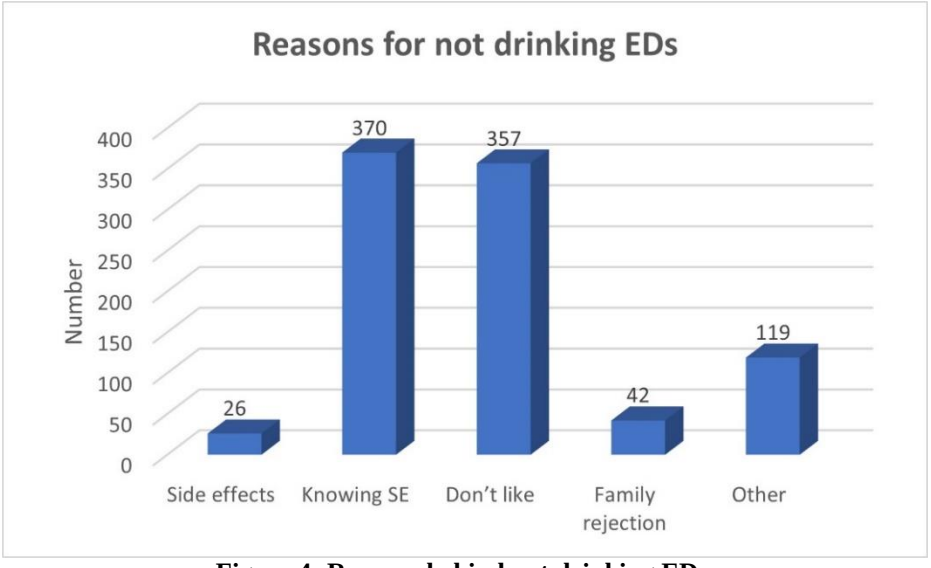

Figure 4: Reasons behind not drinking EDs.

The total knowledge score for participating students ranged from 1 to 17, with a mean ± SD (standard deviation) of 8.37 ± 2.76 and a median of 8. The median score was used to divide the data into two groups: students with a knowledge score less than 8 were classified as having inadequate knowledge, and those with scores equal to or greater than 8 were considered to have adequate knowledge. Hence, the inadequate knowledge group consisted of 693 students (53.4%) while the adequate knowledge group comprised 605 students (46.6%).

Statistically significant differences were found in the total knowledge score across various categories: age,

gender, year of study, residence, weekly income, and ED consumption habits. Higher scores were noted among students older than 23 years of age, males, fifth-year students, those living in urban areas, those receiving a weekly income exceeding $35, and among students who reported using EDs. Among students who indicated not drinking EDs, the group that previously experienced side effects exhibited the highest knowledge scores; the difference between these and other groups was statistically significant. These results are summarized in Table 4.

Table 4: Differences in knowledge scores among different variables

Variable	Total knowledge score	
N= 1298	**Mean ± SD**	***P*-value**
Age[*]		**0.006**[§]
18 – 20 years	7.99 ± 2.51	
21 – 23 years	8.35 ± 2.76	
> 23 years	9.27 ± 2.99	
Gender[**]		**<0.001**[§]
Male	8.82 ± 2.75	
Female	8.07 ± 2.73	
Year of study[*]		**<0.001**[§]
1st year	7.37 ± 2.47	
2nd year	7.99 ± 2.56	
3rd year	8.22 ± 2.60	
4th year	8.69 ± 2.79	
5th year	9.51 ± 2.83	
6th year	9.08 ± 3.60	
Residence[**]		**<0.001**[§]
Urban	8.49 ± 2.76	
Rural	7.80 ± 2.73	
Sleeping regularly[**]		0.990
Yes	8.37 ± 2.79	
No	8.37 ± 2.74	
Sleeping hours[**]		0.196
6 – 8 hours	8.44 ± 2.71	
> 8 hours	8.23 ± 2.86	
Weekly income[*]		**<0.001**[§]
< 20 $	8.13 ± 2.70	
20 – 35 $	8.55 ± 2.72	
> 35 $	9.32 ± 3.03	
Drink EDs		**<0.001**[§]
Yes	9.29 ± 2.67	
No	7.98 ± 2.71	
N = 384		
Frequency of drinking EDs[*]		0.180
Daily	8.95 ± 2.46	
More than once weekly	9.76 ± 2.68	
Once weekly	9.20 ± 2.65	
1 – 3 times monthly	9.13 ± 2.74	
N = 914		
Reasons not to drink[*]		**<0.001**[§]
Experiencing side effects	9.81 ± 2.95	
Knowing the side effects	8.46 ± 2.52	
Disliking EDs	7.53 ± 2.70	
Family disapproval	8.26 ± 2.67	
Others	7.32 ± 2.85	

[*]One-Way ANOVA, [**]Independent-Samples T test, [§]Significant results

DISCUSSION

A review of the literature reveals numerous studies suggesting that EDs can lead to various side effects [7, 25, 26], and in some cases, sudden deaths have been reported [27]. To the best of our knowledge, this study is the first to evaluate the prevalence of ED consumption among medical university students at the University of Mosul. This age group was selected for the study because university students are at high risk for ED consumption, and commercial advertisements for EDs notably target this sector of society. For example, the slogan "Red Bull gives you wings" has become popular among the public [28, 29].

The sample size in the current study was 1,298, compared to 783 in a Saudi study [7], 570 in a Kuwaiti study [29], and 131 in a Polish study [30]. The large sample size in our study may add strength to the generalizability of our results, potentially extending to society in general or specifically to the youth demographic. Similar to our study, a Saudi Arabian study [23] also observed higher participation from female students. This higher rate of female participation reflects the predominance of females opting for careers related to medicine in Arab countries.

The results of the knowledge section displayed some inconsistencies. While the majority of participating students acknowledged knowing what EDs are, fewer could accurately answer specific questions concerning the constituents and effects of EDs. This discrepancy could stem from the characteristic youthful tendency to know about different aspects of life but with a lesser focus on the specific details related to those aspects.

When inquired about their knowledge of ED ingredients, 41.7% of the medical students answered affirmatively, a percentage lower than the reported findings of Cencek et al. [30]. Almost all participants (95.4%) in the present study asserted that EDs could negatively impact health, whereas in Jordan [31] slightly more than 70%, and in Poland [30], only 61.83% of study participants acknowledged these negative effects.

In this study, 30% of participants confirmed they consume EDs. This figure is considerably lower than the 61.8% found in a Polish study conducted by Cencek et al. [30]. This discrepancy could be attributed to differences in sample size. In our research, the sample size was 1,298 students, with 384 reporting ED consumption. In contrast, the Polish study had only 131 participants, with 81 confirmed consumers. Regardless, our results align with other studies conducted among the Marmara University Medical School in Turkey [32] and medical students in South Africa [33]. An Italian study [28] reported that, contrastingly, 22% of participating medical students regularly consumed EDs, and other studies in both Italy [34] and Pakistan [35] found roughly half of the participants were consumers of EDs.

The most common reason cited for ED consumption in our study was the need to increase concentration and improve memory during study and examination periods. This contrasts with a Kuwaiti survey [29] where taste and the need to stay awake were the primary reasons for consuming EDs. Approximately one-third of the participants in our study (35.2%) claimed to consume EDs one to three times monthly, less than the percentage from a study among Saudi students (50.7%) [7]. Most of our study's participants had no preferred time of day for consuming EDs, a finding concurrent with that of Subaiea et al. [7]. These data also align with those reported in the study by Casuccio et al. [28].

In this study, only 23% of students indicated a preference for sugar-free drinks. This proportion mirrors the results obtained by Al-Waalan & Al-Khamees [29], suggesting that sugar-free EDs are not very popular. The inclusion of sugar in these beverages can potentially lead to health issues such as weight gain, which exacerbates the health risks associated with EDs [36]. Almost half of the students (48.7%) preferred the Tiger™ ED brand, followed by Red Bull (24%), mirroring the results of Subaiea et al. [7] in Saudi Arabia regarding Red Bull preferences. In both our study and the Saudi one, taste

emerged as the primary motive for choosing a specific ED brand [7].

Forty-one percent of students in our study reported experiencing side effects after consuming EDs, a figure that aligns with Cencek et al.'s findings [30]. A slightly higher proportion (48%) was noted in a survey by Chuda and Lelonek [37] among medical students in Poland. Insomnia and palpitations were the most commonly reported symptoms in our study, with the latter finding aligning with studies conducted among Polish medical students by Chuda and Lelonek [37] and Semeniuk [38].

Older students and those in their final two years of study exhibited higher knowledge scores than others. This trend may be linked to increased awareness and the curriculum content in medical colleges. Higher knowledge scores were also observed among students who were consumers of EDs. This could be attributed to these individuals handling these beverages more frequently and possibly reading the labels. This is contrary to the results obtained by Subaiea et al. [7], who found an inverse association between knowledge and ED consumption. Our study also found that daily ED consumers showed lower knowledge, a finding consistent with a Saudi survey [7].

Limitations of the study

Since the study design is cross-sectional, it precludes causal interpretation. Additionally, as the results are primarily based on participants' survey responses, there is potential for social desirability bias. These factors represent limitations of this study.

Conclusions

Energy drinks are gaining increasing popularity in recent years, necessitating closer monitoring of their use. Although this study determined that consumption of EDs is relatively low among students at medical colleges, their level of knowledge was also observed to be low. Other concerning findings included a preference for sugar-containing EDs and reports of side effects among consumers. These observations suggest the need for increased governmental oversight of EDs, their targeted advertising campaigns, and their labeling practices. Approaches may include awareness campaigns, promoting healthier alternatives, supporting further research in this area, and working collaboratively with manufacturers to provide beverages with lower sugar content and fewer stimulants.

REFERENCES

1. Reissig CJ., Strain EC., Griffiths RR. Caffeinated energy drinks—a growing problem. *Drug Alcohol Depend*. 2009; 99(1-3):1-10.
2. Ibrahim NKR., Iftikhar R., Murad M., Fida H., Abalkhaeil B., Al Ahmadi J. Energy drinks consumption amongst medical students and interns from three colleges in Jeddah, Saudi Arabia. *J Food Nutr Res*. 2014; 2(4):174-179.
3. Packaged Facts. Energy drinks and shots: US market trends. Rockville, MD: Packaged Facts. Published 2013. https://www.prnewswire.com/news-releases/energy-drinks-and-shots-us-market-trends-190691591.html
4. Nowak D. and Jasionowski A. Analysis of the consumption of caffeinated energy drinks among Polish adolescents. *Int J Environ Res Public Health*. 2015; 12(7):7910-7921.
5. Ehlers A., Marakis G., Lampen A., Hirsch-Ernst KI. Risk assessment of energy drinks with focus on cardiovascular parameters and energy drink consumption in Europe. *Food Chem Toxicol*. 2019; 130:109-121.
6. Breda JJ., Whiting SH., Encarnação R., et al. Energy drink consumption in Europe: a review of the risks, adverse health effects, and policy options to respond. *Front public Heal*. Published online 2014:134.

7. Subaiea GM., Altebainawi AF., Alshammari TM. Energy drinks and population health: consumption pattern and adverse effects among Saudi population. *BMC Public Health*. 2019; 19(1):1-12.
8. Higgins JP., Babu K., Deuster PA., Shearer J. Energy drinks: a contemporary issues paper. *Curr Sports Med Rep*. 2018; 17(2):65-72.
9. Nowak D., Gośliński M., Nowatkowska K. The effect of acute consumption of energy drinks on blood pressure, heart rate and blood glucose in the group of young adults. *Int J Environ Res Public Health*. 2018; 15(3):544.
10. Seifert SM., Schaechter JL., Hershorin ER., Lipshultz SE. Health effects of energy drinks on children, adolescents, and young adults. *Pediatrics*. 2011; 127(3):511-528.
11. Kelsey D., Berry AJ., Swain RA., Lorenz S. A case of psychosis and renal failure associated with excessive energy drink consumption. *Case Rep Psychiatry*. 2019; 2019.
12. Greene E., Oman K., Lefler M. Energy drink–induced acute kidney injury. *Ann Pharmacother*. 2014; 48(10):1366-1370.
13. Shearer J. and Graham TE. Performance effects and metabolic consequences of caffeine and caffeinated energy drink consumption on glucose disposal. *Nutr Rev*. 2014; 72(suppl_1):121-136.
14. Shi X., Xue W., Liang S., Zhao J., Zhang X. Acute caffeine ingestion reduces insulin sensitivity in healthy subjects: a systematic review and meta-analysis. *Nutr J*. 2016; 15(1):1-8.
15. Shanshal SA., Noori AS., Ghazi JA., Dahham AT., Saleh ASM., Al-Qazaz HK. Impact of peptic ulcer disease on the quality of life: A Cross Sectional Study. *Res J Pharm Technol*. 2022; 15(7):3267-3272.
16. Nadeem IM., Shanmugaraj A., Sakha S., Horner NS., Ayeni OR., Khan M. Energy drinks and their adverse health effects: a systematic review and meta-analysis. *Sports Health*. 2021; 13(3):265-277.
17. Silva JGVC., Martins JPG., de Sousa EBG., et al. Influence of energy drinks on enamel erosion: In vitro study using different assessment techniques. *J Clin Exp Dent*. 2021; 13(11): e1076-e1082. doi: 10.4317/jced.57788
18. Saku EY., Nuro-Ameyaw P., Amenya PC., Kpodo FM., Esua-Amoafo P., Kortei NK. Energy drink: the consumption prevalence, and awareness of its potential health implications among commercial drivers in the Ho municipality of Ghana. *BMC Public Health*. 2020; 20(1):1-13.
19. Marczinski CA., Fillmore MT., Bardgett ME., Howard MA. Effects of energy drinks mixed with alcohol on behavioral control: risks for college students consuming trendy cocktails. *Alcohol Clin Exp Res*. 2011; 35(7):1282-1292.
20. Shanshal SA., Al-Sabbagh H., Saadoun M., et al. Energy Drinks: knowledge and Practice Among Non-Medical University Students. *Iraqi J Pharm*. 2023; 20(1):53-59. doi: 10.33899/iphr.2023.139505.1033
21. Aladul MIM., Jamel IA., Thanoon TA., Abd-Alrazzaq FF., Shaker ZS., Jassim TM. Comparison of Job Satisfaction by Alumni and Student Medical Representatives and The Associated Factors in Iraq. *Jordan j. pharm. sci*. 2023; 16(4):785-797.
22. Abazid H., Abutayeh R., Farha RA., Jaffal S., Alkaddah D., Al Jomaa EE. Substance Abuse among University Students: Assessing Prevalence, Risk and Preventive Measures. *Jordan j. pharm. sci*. 2023; 16(2):235-244.
23. Alabbad M., AlMussalam M., AlMusalmi A., et al. Determinants of energy drinks consumption among the students of a Saudi University. *J Fam Community Med*. 2019; 26(1):36. doi: 10.4103/jfcm.JFCM_42_18
24. DeCoster J., Gallucci M., Iselin AM. Best practices for using median splits, artificial categorization, and their continuous alternatives. *J Exp Psychopathol*. 2011; 2(2):197-209. doi: https: //doi.org/10.5127/jep.008310

25. Kozik TM., Shah S., Bhattacharyya M., et al. Cardiovascular responses to energy drinks in a healthy population: the C-energy study. *Am J Emerg Med*. 2016; 34(7):1205-1209.
26. Venkatraman A., Khawaja A., Shapshak AH. Hemorrhagic stroke after consumption of an energy drink. *Am J Emerg Med*. 2017; 35(3):522-e5.
27. Malinauskas BM., Aeby VG., Overton RF., Carpenter-Aeby T., Barber-Heidal K. A survey of energy drink consumption patterns among college students. *Nutr J*. 2007; 6(1):1-7.
28. Casuccio A., Bonanno V., Catalano R., et al. Knowledge, attitudes, and practices on energy drink consumption and side effects in a cohort of medical students. *J Addict Dis*. 2015; 34(4):274-283.
29. Al-Waalan T. and Al Khamees R. Energy Drinks Consumption Patterns Among Young Kuwaiti Adults. In: *SHS Web of Conferences*. Vol 123. EDP Sciences; 2021: 1015.
30. Cencek P., Wawryk-Gawda E., Samborski P., Jodłowska-Jędrych B. Energy drinks-consumption and awareness among students of Medical University of Lublin. *Curr Issues Pharm Med Sci*. 2016;29(4).
31. Thiab S., Barakat M., Nassar RI., et al. Knowledge, attitude, and perception of energy drinks consumption among university students in Jordan. *J Nutr Sci*. 2023; 12: e109. doi: 10.1017/jns.2023.90
32. Hidiroglu S., Tanriover O., Unaldi S., Sulun S., Karavus M. A survey of energy-drink consumption among medical students. *J Pak Med Assoc*. 2013; 63(7):842-845.
33. Lee KH., Human GP., Fourie JJ., Louw WAN., Larson CO., Joubert G. Medical students' use of caffeine for'academic purposes' and their knowledge of its benefits, side-effects and withdrawal symptoms. *South African Fam Pract*. 2009; 51(4):322-327.
34. Oteri A., Salvo F., Caputi AP., Calapai G. Intake of energy drinks in association with alcoholic beverages in a cohort of students of the School of Medicine of the University of Messina. *Alcohol Clin Exp Res*. 2007; 31(10):1677-1680.
35. Aslam HM., Mughal A., Edhi MM., et al. Assessment of pattern for consumption and awareness regarding energy drinks among medical students. *Arch Public Heal*. 2013; 71(1):1-11.
36. Scuri S., Petrelli F., Tesauro M., Carrozzo F., Kracmarova L., Grappasonni I. Energy drink consumption: a survey in high school students and associated psychological effects. *J Prev Med Hyg*. 2018; 59(1):E75.
37. Chuda A. and Lelonek M. A survey of energy drink consumption patterns among 4th and 5th year students of Faculty of Medicine, Medical University of Lodz. *Folia Cardiol*. 2015; 10(3):149-156.
38. Semeniuk W. Consumption of energy drinks among students of the University of Life Sciences in Lublin. *Probl Hig i Epidemiol*. 2011; 92(4):965-968.

المعرفة وممارسة استهلاك مشروبات الطاقة بين طلاب الجامعات الطبية في مدينة الموصل، العراق

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ملخص

الهدف: تحظى مشروبات الطاقة بشعبية كبيرة ويرتبط استهلاكها بآثار ضارة. تهدف هذه الدراسة إلى استكشاف مستوى المعرفة فيما يتعلق بمشروبات الطاقة بين طلاب الطب وممارسات هؤلاء الطلاب الذين يستهلكون هذه المنتجات.

الطرق: تم اعتماد تصميم دراسة مقطعية مستعرضة مبنية على الاستبيان في هذا العمل. تم توزيع المسح على طلاب الكليات الطبية في جامعة الموصل لتقييم معرفة وممارسة هؤلاء الطلاب فيما يتعلق بمشروبات الطاقة.

النتائج: شارك في الدراسة ما مجموعه 1298 طالباً، 60٪ منهم إناث. يعرف معظم الطلاب (89٪) ماهية مشروبات الطاقة، ولكن 42٪ فقط يعرفون مكوناتها. عرف جميع الطلاب تقريبًا (95٪) أن مشروبات الطاقة لها آثار ضارة، لكن القليل منهم فقط يعرفون الآثار المفيدة لهذه المنتجات. اعترف 30٪ فقط من المشاركين بتناول مشروبات الطاقة، لكن أكثر من نصف هؤلاء الطلاب يشربون أقل من 5 علب شهريًا. كانت المنتجات المحتوية على السكر أكثر تفضيلاً من المنتجات الخالية من السكر وكانت تايكر هي العلامة التجارية الأكثر تفضيلاً لمشروبات الطاقة بين الطلاب. أفاد 41 بالمائة من الطلاب الذين يستهلكون مشروبات الطاقة أنهم يعانون من آثار جانبية. كانت معرفة الآثار الجانبية لمشروبات الطاقة هي أكثر الأسباب التي تم الإبلاغ عنها كسبب لعدم شرب هذه المشروبات. تم العثور على الطلاب الأكبر سنًا في السنتين الأخيرتين من الدراسة والطلاب الذين يعيشون داخل المدينة على دراية أكبر بمشروبات الطاقة.

الاستنتاجات: كان مستوى المعرفة بمشروبات الطاقة متدنياً، وهو أمر ينذر بالخطر، خاصة مع تزايد الإقبال على مشروبات الطاقة والحملات الإعلانية الموجهة للشباب.

الكلمات الدالة: مشروبات الطاقة، تايكر، معرفة، استهلاك مشروبات الطاقة، ممارسة.

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Potential Drug-Drug Interactions and their Associated Factors at the University Children's Hospital in Syria: A Cross-Sectional Study

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ABSTRACT

Objective: Polypharmacy among pediatric inpatients is common and exposes children to the risk of drug-drug interactions (DDIs). This study aimed to characterize potential DDIs (pDDIs) and their associated risk factors among pediatric inpatients.

Methods: A cross-sectional study was conducted over six months at the University Children's Hospital in Damascus. A total of 575 children taking two drugs or more participated. pDDIs were checked using Lexi-Interact® software. pDDIs within risk category B (No action needed), C (Monitor therapy), D (Modify regimen), and X (Avoid combination) were included. Logistic regression was used to identify factors associated with pDDIs.

Results: At least one pDDI was detected in 49.7% of children. Overall, 744 pDDIs were identified. The majority of pDDIs were within risk category C (71.6%), followed by D (14%), B (12.8%), and X (1.6%). The most common pDDIs were: aminoglycosides - penicillins (n=56), aminoglycosides - cephalosporins (n=27), and vitamin D analogs - calcium salts (n=23). The number of prescribed drugs and nervous system drugs were significantly associated with the presence of pDDIs.

Conclusion: pDDIs among pediatric inpatients were prevalent. The majority of the pDDIs were within risk category C, which necessitates therapy monitoring and necessary action to avoid adverse consequences.

Keywords: Drug-drug interactions, pediatrics, pediatric inpatients, drug safety, Syria.

INTRODUCTION

Pharmacotherapy in children requires special interest due to differences in both pharmacokinetics and pharmacodynamics and insufficient evaluation of the use of many drugs compared to adults. Pediatric polypharmacy is defined as the concurrent use of two or more medications [1]. Polypharmacy is common among pediatric patients, with a higher prevalence in inpatient settings compared to outpatient settings, likely due to the complexity of children's health conditions in hospitals [1]. While polypharmacy may be necessary for pediatric disease management, it does expose children to the risk of drug-drug interactions (DDIs). According to previously published studies, potential drug-drug interactions (pDDIs) are prevalent among pediatric inpatients [2].

pDDIs are a risk factor for the occurrence of adverse drug reactions (ADRs) [3,4,5]. Data on the contribution of pDDIs to the development of ADRs in pediatrics is scarce, but a higher number of prescribed medicines have been found to lead to a higher rate of ADRs in children. DDIs are a possible explanation for this association [6,7]. Additionally, pDDIs were associated with longer stays in pediatric intensive care units [8]. Moreover, 57% of ADRs detected in children in neuropsychiatric units were due to DDIs [9].

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Detecting and understanding pDDIs is fundamental in preventing their adverse consequences. So far, pDDIs among pediatric inpatients in Syria have not been investigated. Real-world data would enhance the awareness of physicians and pharmacists about pDDIs and their significance in children.

OBJECTIVES

The current study aimed to assess the frequency, types, and risk factors of pDDIs among children in the largest children's hospital in Syria.

METHODS

Study design and setting

A cross-sectional study was conducted over a six-month period between March and September 2018 in the public pediatric ward at the University Children's Hospital (UCH) in Damascus. UCH in Damascus is a public teaching hospital and the largest pediatric hospital in Syria. The public pediatric ward includes 116 beds and provides care for children with various diseases including cardiac, neurological, gastrointestinal, orthopedic, renal, and metabolic diseases. UCH does not have a clinical pharmacist on-site.

Ethical considerations

This study was approved by the Scientific Research Council at the Arab International University (Decision No. 7/6, August 16, 2017). Administrative permission was obtained from the hospital for access to patients' charts and for conducting analyses.

Data source

Prescriptions for admitted children were collected once a week. As children were divided according to their diseases and ages into 22 rooms, stratified random sampling was utilized to select patients from every room in a ratio of 1:2:3 for rooms occupied by 1-3, 4-5, and 6-8 patients respectively. If distinct prescriptions were found for the same patient/admission, only one prescription was selected for inclusion in the sample. Children prescribed fewer than two medications were excluded from the study.

The following demographic data were collected from the patient charts for each child: age, gender, length of stay, and the number of prescribed drugs. Children were divided into the following age groups based on the International Conference on Harmonisation (ICH) E11 classifications [10]:

- Newborn infants (0–28 days)
- Infants and toddlers (>28 days–23 months)
- Preschool children (2–5 years)
- School age children (6–11 years)
- Adolescents (12–16 years)

Data on all prescribed drugs, with the exception of sodium chloride and glucose intravenous solutions, were collected. Drugs were classified according to the first level of the Anatomical Therapeutic Chemical (ATC) classification system [11].

Screening for DDIs

Drug interactions were checked using Lexi-Interact® software, a product of Wolters Kluwer. Lexi-Interact® is an online software that has demonstrated high sensitivity and specificity [12,13]. Detected pDDIs were classified according to risk rating, severity, and reliability level as indicated by the software.

Lexi-Interact® assigns DDIs with risk rates of A, B, C, D, and X according to the action required to manage the DDI, as follows: A: No known interaction, B: No action needed, C: Therapy monitoring is recommended, D: Therapy modification is considered, and X: The combination should be avoided. DDIs with a risk rating of A were not considered in the analysis of this study.

Regarding severity level, DDIs are classified by Lexi-Interact® into: Major (the interaction may be life-threatening or cause permanent damage), Moderate (the patient's condition may deteriorate, requiring additional care or extended hospitalization), and Minor (the interaction is not medically detrimental).

Additionally, the dependability of the DDIs is categorized by the software based on the quality and quantity of supporting medical literature. This is broken down into: Excellent, Good, Fair, and Poor.

Statistical analysis

Descriptive statistics were used to analyze the population characteristics and the pDDIs.

Univariate and multivariate binary logistic regression analyses were performed to assess the factors potentially associated with pDDIs. The occurrence of a pDDI was the dependent variable. The predictor variables tested included: gender, age, duration of stay, and the number of prescribed medicines. ATC codes prescribed for ≥10% of the patients were also included. Variables with a univariate P-value <0.1 were included in the multivariate analysis. Predictor variables in the multivariate analysis with a P-value of ≤0.05 were considered statistically significant. All statistical analyses were performed using IBM SPSS v22.

RESULTS

Study population and prescriptions data

A total of 577 children admitted to the public pediatric ward between March and September 2018 were included in the study. Figure 1 illustrates the data collection flow throughout the study.

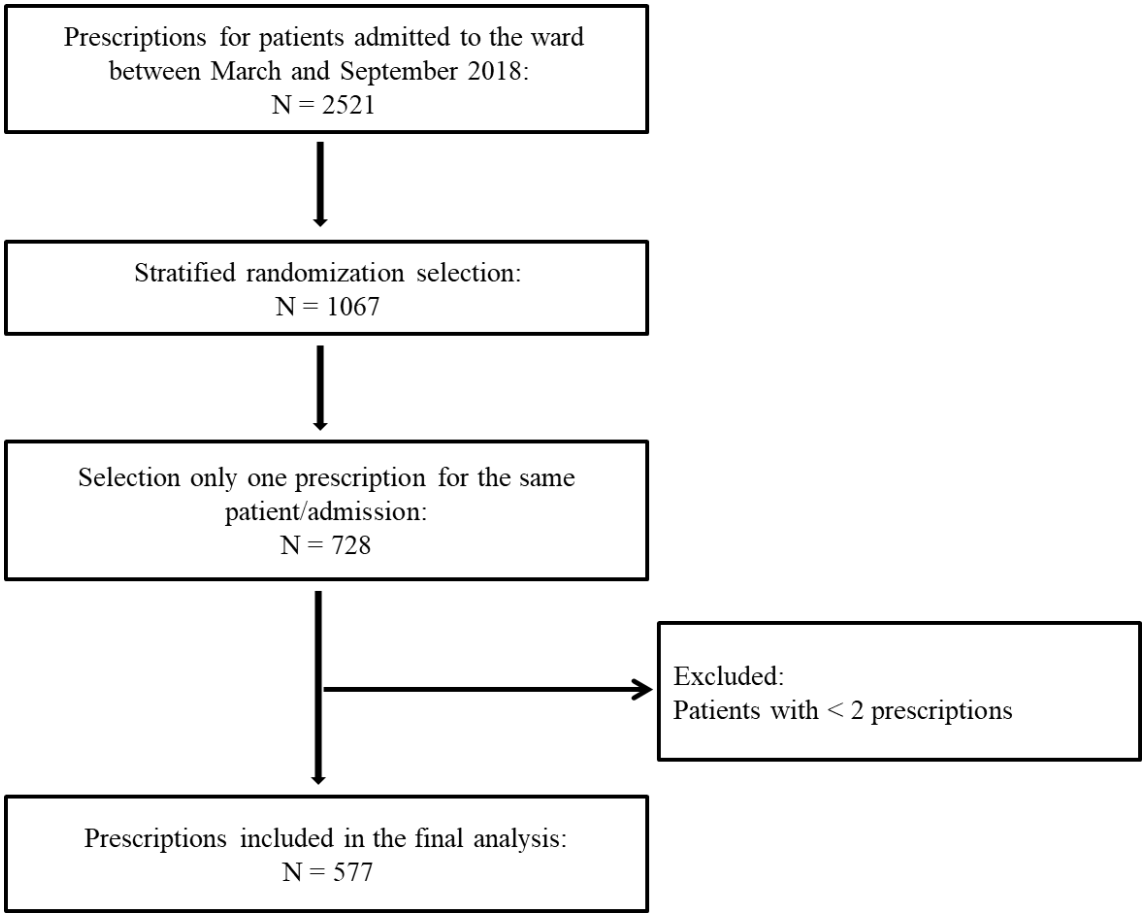

Figure 1: Data collection flow

The demographic and clinical characteristics of the children are presented in Table 1. Out of the study population, 328 (56.8%) were boys. The median age was 1.5 years, with a range from one day to 14 years old. The

largest proportion of the population were infants and toddlers, accounting for 313 (54.2%) of the subjects. The median duration of stay in the hospital was ten days, ranging from one to 133 days.

Table 1: Demographic and clinical characteristics of patients (N=577)

Characteristics	Value
Age (year), median (range)	1.5 (0-14)
Gender, n (%)	
Male	328 (56.8%)
Female	249 (43.2%)
Age groups, n (%)	
Newborn infants	5 (0.9%)
Infants and toddlers	313 (54.2%)
Pre-school children	120 (20.8%)
School age children	117 (20.3)
Adolescents	22 (3.8%)
Duration of stay in the hospital, median (range)	10 (1-133)
Duration of stay in the hospital, n (%)	
1-3	74 (12.8%)
4-7	116 (20.1%)
≥ 8	387 (67.1%)
Prescribed medication per patient, median (range)	4 (2-14)
Prescribed medication per patient, n (%)	
2-4	358 (62%)
5-9	192 (33.3%)
≥ 10	27 (4.7%)
ATC code, n (%)	
ATC code "A" (Alimentary tract and metabolism)	360 (62.4%)
ATC code "B" (Blood and blood forming organs)	147 (25.5%)
ATC code "C" (Cardiovascular system)	122 (21.1%)
ATC code "D" (Dermatologicals)	30 (5.2%)
ATC code "H" (Systemic hormonal preperations)	133 (23.1%)
ATC code "J" (Antiinfectives for systemic use)	487 (84.4%)
ATC code "L" (Antineoplastic and immunomodulating agents)	6 (1%)
ATC code "M" (Musculo-skeletal system)	55 (9.5%)
ATC code "N" (Nervous system)	236 (40.9%)
ATC code "P" (Antiparasitic products, insecticides and repellents)	2 (0.3%)
ATC code "R" (Respiratory system)	30 (5.2%)
ATC code "S" (Sensory organs)	17 (2.9%)
ATC code "V" (Various)	5 (0.9%)

In total, 2,587 drugs were prescribed with a median of four drugs per patient, ranging from two to fourteen drugs. The largest proportion of children, 358 (62%), received between two and four drugs. Drugs with ATC code "J" (Antiinfectives for systemic use) had the highest prescription rate, with 84.4% of children receiving at least one drug with an ATC code of "J".

Characteristics of pDDIs

There were 287 children (49.7%) who had at least one pDDI. In total, 744 pDDIs were identified. Among patients with pDDIs, the median number of pDDIs per patient was two, ranging from one to fifteen. The majority of detected pDDIs were of risk rate C (71.6%). The distribution of pDDIs according to their risk rating is presented in Table 2.

Table 2: Distribution of pDDIs according to their risk rating

Risk rate	Number of pDDIs (%)
B	95 (12.8%)
C	533 (71.6%)
D	104 (14.0%)
X	12 (1.6%)

The majority of the detected pDDIs, 568 (76.4%), had moderate severity, while 101 (13.6%) and 74 (9.9%) had minor and major severity respectively.

The most common pDDIs were aminoglycosides with penicillins (56 instances), aminoglycosides with cephalosporins (27 instances), and vitamin D analogs with calcium salts (23 instances).

The most common pDDIs in each risk category are presented in Table 3.

Factors associated with pDDIs

The association between patient characteristics and the occurrence of pDDIs in both univariate and multivariate analyses is presented in Table 4. According to the univariate model, the exposure to pDDIs was significantly associated with the number of prescribed drugs and ATC codes "B" (Blood and blood-forming organ drugs), "C" (Cardiovascular system drugs), "J" (Antiinfectives for systemic use), "H" (Systemic hormonal preparations), and "N" (Nervous system drugs).

The multivariate model indicated a significant association between the number of prescribed drugs and the presence of pDDIs. The adjusted odds ratio (AOR) increased from 5.80 (95% CI 3.64-9.26) in patients prescribed between five and nine drugs to 35.86 (95% CI 4.52-284.69) in patients prescribed ≥10 drugs. Patients prescribed drugs with ATC code "N" (Nervous system drugs) had a higher risk of having a pDDI (AOR=2.82, 95% CI 1.87-4.27).

Table 3: The most commonly detected pDDIs in each risk category

pDDI	No.	Severity	Level of evidence	clinical effect
Risk rate X				
VitD analogs - VitD analogs	5	Moderate	Fair	↑ Adverse/toxic effect of VitD
Carbamazepine - Linezolide	3	Major	Fair	Risk of serotonin syndrome
Domperidone - Fluconazole	2	Major	Fair	↑ QTc-prolonging effect, ↑ Serum level of domperidone
Domperidone - Itraconazole	1	Major	Fair	↑ Serum level of domperidone
Gentamicin - Amikacin	1	Moderate	Good	↑ Nephrotoxicity and/or neurotoxicity
Risk rate D				
Dexamethasone - Phenytoin	16	Major	Fair	↓ Serum level of dexamethasone
Aminoglycosides - Vancomycin	14	Moderate	Fair	↑ Nephrotoxicity and/or neurotoxicity
Al/Mg hydroxide - Corticosteroids	9	Moderate	Fair	↓ Bioavailability of oral corticosteroids
Carbapenems - Valproic acid	5	Major	Good	↓ serum level of valproate products
Calcium carbonate - Prednisolone	4	Moderate	Fair	↓ Bioavailability of oral prednisolone
Calcium Salts - Levothyroxine	4	Moderate	Fair	↓ Therapeutic effect of levothyroxine
Risk rate C				
Aminoglycosides - Penicillins	56	Moderate	Excellent	↓ Serum level of aminoglycosides
Aminoglycosides - Cephalosporins	27	Moderate	Excellent	↑ Nephrotoxic effect of aminoglycosides, ↓ Serum level of aminoglycosides
VitD analogs - Calcium Salts	23	Moderate	Fair	↑ Adverse/toxic effect of VitD
Digoxin - Furosemide	17	Moderate	Fair	↑ Adverse/toxic effect of digoxin
Allopurinol - Furosemide	15	Moderate	Fair	↑ Adverse/toxic effect of allopurinol
Amlodipine - Calcium salts	15	Moderate	Excellent	↓ Therapeutic effect of amlodipine
Risk rate B				
Ondansetron - Paracetamol	17	Minor	Fair	↓ Analgesic effect of paracetamol
Iron - VitE	11	Minor	Fair	↓ Therapeutic effect of iron
Ciprofloxacin - Fluconazole	7	Minor	Fair	↑ QTc-prolonging effect
Aminoglycosides - Clindamycin	5	Minor	Poor	↑ Nephrotoxic effect of aminoglycosides
Metronidazole - Ondansetron	5	Minor	Fair	↑ QTc-prolonging effect

Table 4: Risk factors associated with potential drug–drug interactions

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	AOR (95% CI)	P value
Gender				
Female	Reference			
Male	1.12 (0.80-1.55)	0.517		
Age groups				
Newborn infants	Reference			
Infants and toddlers	0.60 (0.10-3.63)	0.576		
Pre-school children	0.60 (0.10-3.74)	0.587		
School age children	0.99 (0.16-6.17)	0.994		
Adolescents	0.46 (0.06-3.35)	0.444		
Number of prescribed drugs				
2-4	Reference		Reference	
5-9	7.95 (5.28-11.96)	<0.001*	5.80 (3.64-9.26)	<0.001*
≥10	57.86 (7.75-431.72)	<0.001*	35.86 (4.52-284.69)	0.001*
Duration of stay (days)				
1-3	Reference			
4-7	1.33 (0.74-2.39)	0.338		
≥8	1.25 (0.76 – 2.06)	0.383		
ATC code "A" (Alimentary tract and metabolism)	1.30 (0.93-1.83)	0.125		
ATC code "B" (Blood and blood forming organs)	1.61 (1.10-2.35)	0.014*	1.15 (0.71-1.88)	0.573
ATC code "C" (Cardiovascular system)	3.02 (1.96-4.64)	<0.001*	1.69 (0.97-2.94)	0.066
ATC code "J" (Antiinfectives for systemic use)	1.88 (1.18-2.96)	0.008*	1.31 (0.76-2.25)	0.331
ATC code "H" (Systemic hormonal preperations)	2.51 (1.67-3.78)	<0.001*	1.59 (0.96-2.63)	0.070
ATC code "N" " (Nervous system)	2.76 (1.96-3.89)	<0.001*	2.82 (1.87-4.27)	<0.001*

AOR adjusted odds ratio, OR odds ratio

* P-value is statistically significant

DISCUSSION

This study is the first to characterize pDDIs among pediatric inpatients in Syria. Nearly half of the children in the study had at least one pDDI. The majority of detected pDDIs fell within risk category C (71.6%). Both the number of prescribed drugs and receiving drugs with an ATC code of "N" (Drugs for nervous system) were significantly associated with the occurrence of pDDIs.

Almost half of the children (49.7%) had at least one pDDI. This is similar to the results of previous studies, wherein 42% to 52.3% of pediatric inpatients were found to have at least one pDDI [1,14,15,16]. However, the percentage of children exposed to pDDIs in our study was much higher compared to that in Langerová et al.'s study (3.83%)[17]. This difference might be partially due to variations in the inclusion criteria and the software used to detect pDDIs. Langerová et al. utilized the INFOPHARM Drug Interactions Compendium® computer program in

their study. An evaluation of INFOPHARM's performance could not be found in the literature. On the other hand, Lexi-Comp, which was used in our study, has been found to have a higher sensitivity compared to many other drug-drug interaction software programs [13].

The majority of detected pDDIs in our study (71.6%) were within risk category C, followed by risk categories D (14.0%), B (12.8%), and X (1.6%). This differed from the results of Bebitoğlu et al., who used the same DDI checker [16]. According to Bebitoğlu's study, 44.8% and 42.7% of pDDIs were within risk categories B and A respectively, whereas 8.4% and 4.1% of the pDDIs were classified under risk categories C and D respectively. No pDDIs were detected in risk category X.

This discrepancy might be partially explained by differences in the inclusion/exclusion criteria of the two studies. pDDIs within risk category A were excluded from our study, whereas 42.7% of the pDDIs in Bebitoğlu's study fell into risk category A. Additionally, vitamins were excluded from the analysis in Bebitoğlu's study, whereas they were included in our study and were often involved in pDDIs under risk category C, such as Vitamin D analogs with calcium salts.

Furthermore, the medical conditions of children in our study were likely more complex compared to those in Bebitoğlu et al.'s study. This is indicated by the longer length of stay in our study (ten days versus 5.1±2.0 days in Bebitoğlu et al.'s study), and a much higher proportion of children who were prescribed ≥5 medications (38% versus 5.6%).

Consequently, children in our study were prescribed different medications and combinations. For instance, to treat infections, children were often prescribed combinations such as aminoglycosides with penicillins, aminoglycosides with cephalosporins, and aminoglycosides with vancomycin. These largely contributed to the proportion of pDDIs under risk categories C and D.

The most common pDDI in our study involved a combination of aminoglycosides and penicillins, which have a moderate severity and fall into risk category C. The interaction between aminoglycosides and penicillins, which may decrease the serum concentration of aminoglycosides, has also been reported as a common pDDI in previous pediatric studies [18,19,20]. This interaction is particularly significant when penicillin and aminoglycoside are in contact over a prolonged period of time, such as in patients with renal dysfunction. In this case, treatment monitoring is recommended, especially in severely ill patients [21].

The second most common pDDI was between aminoglycosides and cephalosporins, which was also found to have moderate severity and to fall into risk category C. This pDDI has also been among the most common pDDIs detected in previous pediatric studies [22,23]. The combination of aminoglycosides and cephalosporins is synergistically nephrotoxic, necessitating careful monitoring for signs of nephrotoxicity [24,25]. Additionally, cephalosporins may inactivate aminoglycosides [26]. However, this is expected to be clinically significant only in patients with severely impaired renal function [27,28].

The third most common pDDI was between vitamin D analogs and calcium salts. The severity of this pDDI is moderate and it falls into risk category C, requiring monitoring of serum calcium concentrations and signs/symptoms of hypercalcemia.

In this study, both the number of prescribed drugs and ATC code "N" (Drugs for nervous system) were found to be significantly associated with the presence of pDDIs. The number of prescribed drugs has previously been identified as a risk factor for the presence of pDDIs in pediatric wards [14,17]. Studies assessing the association between medication groups and the presence of pDDIs among children are scarce. However, Langerová et al. found a significant association between antiepileptic drugs (ATC code: N03) and the presence of pDDIs among pediatric inpatients [17].

Although a high proportion of children in this study were exposed to pDDIs, the majority of pDDIs fell within risk category C (71.6%) and were of moderate severity (76.4%). This implies that most pDDIs are not expected to be life-threatening or cause permanent damage. However, additional care and therapy monitoring are required, especially in the presence of risk factors or other pDDIs with similar potential adverse effects. According to published literature, not all pDDIs result in actual DDIs or ADRs, but life-threatening and fatal ADRs can develop [29]. Therefore, pediatricians should be informed about pDDIs and a plan should be developed at the hospital level to avoid the adverse effects of pDDIs.

It is important to draw the attention of clinicians to the significant association between the occurrence of pDDIs, the number of prescribed drugs, and the prescribing of drugs for the nervous system. We also suggest a protocol be developed to manage the most frequently detected pDDIs in all risk categories each time the involved combination is prescribed. However, screening for pDDIs with every prescription of more than one drug is recommended to detect less common pDDIs and to avoid adverse consequences. This could be better achieved with the support of clinical pharmacists. Clinical pharmacists are qualified to perform medication reviews, detect drug-related problems including DDIs, and intervene to avoid negative impacts on patients [30,31].

A limitation of this study is that the clinical outcomes of the pDDIs were not evaluated. Furthermore, the drug interaction checker used in the study detects pDDIs between each pair of drugs without considering the influence of additional drugs on the pDDIs. As a result, the severity of some pDDIs may have been underestimated. Another limitation is the single-center design of the study, which limits the generalizability of its findings. Moreover, both newborn infants and adolescents were not sufficiently represented in the study, due to the small percentages of these age groups in the sample. Future research to assess actual DDIs in multiple pediatric settings, including different age groups, is needed.

CONCLUSION

Almost half of the pediatric inpatients in our study had at least one pDDI. The number of drugs and drugs with ATC code "N" (Drugs for nervous system) showed a significant association with the presence of pDDIs. Clinicians at the hospital are advised to exercise caution when prescribing for patients with these risk factors to detect and assess any potential pDDIs. The majority of the detected pDDIs fell within risk category C, necessitating therapy monitoring and nimble response as needed to avoid adverse consequences. Future investigations to evaluate the actual impact of pDDIs on pediatric inpatients are recommended.

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REFERENCES

1. Baker C., Feinstein J. A., Ma X., Bolen S., Dawson N. V., Golchin N. et al. Variation of the prevalence of pediatric polypharmacy: A scoping review. *Pharmacoepidemiol Drug Saf*. 2019; 28(3):275-287.
2. Feinstein J., Dai D., Zhong W., Freedman J. and Feudtner C. Potential drug-drug interactions in infant, child, and adolescent patients in children's hospitals. *Pediatrics*. 2015; 135(1):e99-108.
3. Obreli Neto P. R., Nobili A., Marusic S., Pilger D., Guidoni C. M., Baldoni Ade O. et al. Prevalence and predictors of potential drug-drug interactions in the elderly: a cross-sectional study in the brazilian primary public health system. *J Pharm Pharm Sci*. 2012; 15(2):344-354.
4. De Paepe P., Petrovic M., Outtier L., Van Maele G. and Buylaert W. Drug interactions and adverse drug reactions in the older patients admitted to the emergency department. *Acta Clin Belg*. 2013; 68(1):15-21.
5. Ray S., Pramanik J., Bhattacharyya M. and Todi S. Prospective observational evaluation of incidences and implications of drug-drug interactions induced adverse drug reactions in critically ill patients. *Indian J Pharm Sci*. 2010; 72(6):787-792.
6. Sugioka M., Tachi T., Mizui, T., Koyama A., Murayama A., Katsuno H. et al. Effects of the number of drugs used on the prevalence of adverse drug reactions in children. Sci Rep 2020; 10: 21341.
7. Lima E.C, Camarinha B.D., Ferreira Bezerra N.C., Panisset A.G., Belmino de Souza R., Silva M.T. et al. Severe Potential Drug-Drug Interactions and the Increased Length of Stay of Children in Intensive Care Unit. *Front Pharmacol.* 2020; 11:555407.
8. Rashed A.N., Wong I.C., Cranswick N., Tomlin S., Rascher W. and Neubert A. Risk factors associated with adverse drug reactions in hospitalised children: international multicentre study. *Eur J Clin Pharmacol*. 2012;68(5):801-10.
9. Giurin M. S., Trojniak M. P., Arbo A., Carrozzi M., Abbracciavento G., Monasta L. et al. Safety of Off-Label Pharmacological Treatment in Pediatric Neuropsychiatric Disorders: A Global Perspective from an Observational Study at an Italian Third Level Children's Hospital. *Front. Pharmacol*. 2022; 12;13:837692.
10. EMA. ICH Topic E11. Clinical investigation of medicinal products in the pediatric population. CPMP/ICH/2711/99. 2001. https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-1.pdf. Accessed 23.11.2022.
11. WHO Collaborating Centre for Drug Statistics Methodology. ATC classification index with DDDs. Oslo, Norway 2021. 2022. Available at https://www.whocc.no/atc_ddd_index/. Accessed 23.11.2022.
12. Reis A. M. M. and Cassiani S. H. D. B. Evaluation of three brands of drug interaction software for use in intensive care units. *PharmWorld Sci*. 2010; 32(6):822–828.
13. Vonbach P., Dubied A., Krähenbühl S. and Beer J. H. Evaluation of frequently used drug interaction screening programs. *Pharm World Sci*. 2008; 30(4):367–374.
14. Getachew H., Assen M., Dula F. and Bhagavathula A. S. Potential drug–drug interactions in pediatric wards of Gondar University Hospital, Ethiopia: A cross sectional study. *Asian Pac J Trop Biomed*. 2016; 6(6):534-538.
15. Nawaz H. A., Khan T. M., Adil Q., Goh K. W., Ming L. C., Blebil A. Q. et al. A Prospective Study of Medication Surveillance of a Pediatric Tertiary Care Hospital in Lahore, Pakistan. *Pediatr Rep*. 2022; 14(2):312-319.
16. Bebitoğlu B. T., Oğuz E., Nuhoğlu Ç., Dalkılıç A. E. K., Çirtlik P., Temel F. et al. Evaluation of potential drug-drug interactions in a pediatric population. *Turk Pediatri Ars*. 2020; 55(1):30-38.

17. Langerová P., Prokeš M., Konvalinka M., Fürstová J. and Urbánek K. Incidence of potential drug interactions in medication prescriptions for children and adolescents in the University Hospital Olomouc, Czech Republic. *Eur J Pediatr*. 2013; 172(5):631-638.
18. Dai D., Feinstein J. A., Morrison W., Zuppa A. F. and Feudtner C. Epidemiology of Polypharmacy and Potential Drug-Drug Interactions Among Pediatric Patients in ICUs of U.S. Children's Hospitals. *Pediatr Crit Care Med*. 2016; 17(5):e218-e228.
19. Qorraj-Bytyqi H., Hoxha R., Krasniqi S., Bahtiri E. and Kransiqi V. The incidence and clinical relevance of drug interactions in pediatrics. *J Pharmacol Pharmacother*. 2012; 3(4):304-307.
20. Martinbiancho J, Zuckermann J, Dos Santos L, Silva MM. Profile of drug interactions in hospitalised children. *Pharm Pract (Granada)*. 2007; 5(4):157-161.
21. Farchione L. A. Inactivation of aminoglycosides by penicillins. *Journal of Antimicrobial Chemotherapy*. 1981; 8, Suppl A: 27-36.
22. Rao C., Shenoy V. and Udaykumar P. Potential Drug–Drug Interactions in the Pediatric Intensive Care Unit of a Tertiary Care Hospital. *Journal of Pharmacology and Pharmacotherapeutics*; 2019; 10(2):63-68.
23. Tavousi F., Sadeghi A., Darakhshandeh A. and Moghaddas A. Potential Drug-drug Interactions at a Referral Pediatric Oncology Ward in Iran: A Cross-sectional Study. *J Pediatr Hematol Oncol*. 2019; 41(3):e146-e151.
24. Mannion J. C., Bloch R. and Popovich N. G. Cephalosporin-aminoglycoside synergistic nephrotoxicity: fact or fiction? *Drug Intell Clin Pharm*. 1981; 15(4):248-56.
25. Rankin G. O. and Sutherland C. H. Nephrotoxicity of aminoglycosides and cephalosporins in combination. *Adverse Drug React Acute Poisoning Rev*. 1989; 8(2):73-88.
26. Wright D. N., Marble D. A., Saxon B., Johnson C. C., Bosso J. A. and Matsen J. M. In vitro inactivation of aminoglycosides by cephalosporin antibiotics. *Arch Pathol Lab Med*. 1988; 12(5):526-8.
27. Flynn Pharma Ltd. Tobramycin 40 mg/ml solution for injection vials SmPC. 2022. https://www.medicines.org.uk/emc/product/10194/smpc. Accessed 03.02.2023.
28. Neon Healthcare Ltd. Amikacin 250mg/ml solution for injection/infusion SmPC. 2022. Available at: https://www.medicines.org.uk/emc/product/14189/smpc. Accessed 16.03.2023.
29. Magro L., Moretti U. and Leone R. Epidemiology and characteristics of adverse drug reactions caused by drug-drug interactions. *Expert Opin Drug Saf*. 2012;11(1):83–94.
30. Abu-Oliem1 A. S., Al-Sharayri M. G., AlJabra R. J. and Hakuz N. M. A Clinical Trial to Investigate the role of Clinical Pharmacist in Resolving/Preventing Drug Related Problems in ICU Patients Who Receive Anti-infective Therapy. *Jordan j. pharm. sci*. 2013; 6(3):292-8.
31. Issa A., Abu Farha R., Elayeh E. and Bustanji Y. The Impact of Lack of Pharmacist Contribution on the Prescription Patterns and the Appropriateness of Indications of NSAIDs, A Cross-Sectional Study. *Jordan j. pharm. sci*. 2013; 6(2):258-69.

التداخلات الدوائية المحتملة والمتنبّئات المرتبطة بحدوثها في مشفى الأطفال الجامعي في سورية: دراسة مقطعية

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ملخص

هدف الدراسة: إعطاء الأدوية المتعددة للأطفال المرضى ممارسة شائعة وتُعرّض الأطفال لخطر التداخلات الدوائية (DDIs). تهدف هذه الدراسة إلى توصيف الـ DDIs المحتملة (pDDIs) وعوامل الخطورة المرتبطة بها لدى الأطفال المقبولين في المستشفى.

المنهج البحثي: أجريت دراسة مقطعية لمدة 6 أشهر في مشفى الأطفال الجامعي بدمشق. ضمّت الدراسة 577 طفلاً يتلقون دواءين أو أكثر. استُخدم برنامج Lexi-Interact® لتحرّي pDDIs. شمل التحليل الـ pDDIs من الفئات: B (لا حاجة لاتخاذ إجراء)، C (مراقبة المعالجة)، D (تعديل الجرعات)، وX (تجنب المشاركة). استُخدم الانحدار اللوجستي لتحديد عوامل الخطورة المرتبطة بـ DDIs.

النتائج: تم الكشف عن وجود pDDI واحد على الأقل لدى 49.7% من الأطفال. بلغ عدد الـ pDDIs ما مجموعه 744 تداخلاً دوائياً. تنتمي معظم الـ pDDIs إلى فئة الخطورة C (71.6%)، تليها التداخلات من الفئة D (14%)، B (12.8%) وX (1.6%). أكثر الـ pDDIs تكراراً: الأمينوغليكوزيدات – البنسلينات (56 مشاركة)، الأمينوغليكوزيدات – السيفالوسبورينات (27 مشاركة) و فيتامين د – أملاح الكالسيوم (23 مشاركة). شملت العوامل المؤثرة على وجود pDDIs كلاً من عدد الأدوية الموصوفة ووصف دواء من زمرة الأدوية العصبية.

الاستنتاجات: معدّل الـ pDDIs لدى الأطفال في المشفى مرتفع. معظم الـ pDDIs من فئة الخطورة C، مما يستدعي مراقبة العلاج واتخاذ الإجراء المناسب لتجنب التبعات الضارة.

الكلمات الدالة: التداخلات الدوائية، طب الأطفال، الأطفال في المشفى، مأمونية الدواء، سورية.

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MMP-1 and MMP-7 Expression is Influenced by Ginsenosides in Mice Exposed to Aflatoxin B1: *in vivo* Study

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ABSTRACT

Panax ginseng (PG), one of the most widely used herbal medicines, has demonstrated various beneficial effects such as anti-inflammatory, antioxidant, and anticancer impacts. Naturally occurring ginsenosides in the ginseng plant inhibit cell proliferation and significantly reduce liver damage induced by certain chemicals. Aflatoxin B1 (AFB1) is a primary mycotoxin due to its hepatotoxic, immunotoxic, and oncogenic effects in animal models and humans. In this study, we examined the effects of assorted doses of PG aqueous crude extract on the expression of matrix metalloproteinase 1 and 7 (MMP-1 and MMP-7) in the kidney, spleen, and liver of experimental AFB1-exposed mice, using immunohistochemistry (IHC). Mice were orally administered 6 mg/kg body weight (bw) of refined AFB1 (isolated and extracted from Aspergillus flavus, conc. 0.05 ppm) twice weekly for two weeks. We then compared the effects of three different doses (50, 100, and 150 mg/kg bw) of crude ginseng. We estimated the expression of MMP-1 and 7 in organs using IHC. We used the 6 mg/kg of purified AFB1, representing a 60% concentration, as a control group. IHC analysis showed that MMP (1 and 7) expression in the spleen, liver, and kidney of mice decreased after treatment with ginseng crude extract. MMP-1 expression was reduced in the liver by approximately 2.6 times, while the effectiveness in the MMP-1 reduction reached 9 and 8 times, respectively, in the spleen and kidney when treated with a higher dose of PG compared to the control. MMP-7 expression was reduced in the liver by approximately 13 times, while the reduction effectiveness fell to 2.3 and 5.6 times in the spleen and kidney when treated with a higher dose of PG compared to the control. The reduction in MMPs expression due to the effect of PG aqueous crude extract was observed to act against the effect of AFB1 on various living organs involved in AFB1 metabolism. IHC analysis indicated a more significant reduction efficiency observed in the expression of MMP-7 compared to both studied markers in the mice's liver.

Keywords: Ginseng, AflatoxinB1, Matrix metalloproteinase.

INTRODUCTION

Ginseng refers to the dried roots of several plants of the species Panax sp. (Family Araliaceae). The three major commercial ginsengs are Panax ginseng Meyer (Korean ginseng), which has been used as an herbal medicine for more than 2000 years, Panax quinquifolium L. (American ginseng), and Panax notoginseng (Chinese ginseng) [1]. These ginsengs have a long history of use as food or medicinal products in China, Korea, Japan, and other Asian countries. Ginseng is well-known as an adaptogen and a

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restorative tonic and is widely used in Traditional Chinese Medicine (TCM) and Western herbal preparations [2]. The therapeutic potential and antioxidant properties of ginseng have been widely explored. Research suggests that ginseng may possess anti-inflammatory properties, potentially beneficial for managing conditions related to chronic inflammation, and for regulating blood pressure, metabolism, and immune system function [3,4]. Furthermore, ginseng treatment can reduce the severity of histopathological, histochemical, and serological alterations, but it does not completely eliminate them [5,6]. Ginsenosides are secondary ginseng metabolites, representing the most critical components of ginseng, and are involved in the modulation of various cellular functional activities.

Aflatoxins (AFs) are secondary metabolites predominantly produced by Aspergillus fungi. Approximately 20 types of AFs, produced by Aspergillus flavus and Aspergillus parasiticus, have been detected in food and feed [7,8]. Aflatoxin B1 (AFB1) is a highly hazardous mycotoxin with potent carcinogenic and mutagenic properties. The presence of aflatoxins in food and feed leads to significant physiological effects such as reduced white and red blood cell counts, hemoglobin, hematocrit, alanine aminotransferase activity, and body and organ weight [9,10]. Subpar food and feed processing and storage conditions can allow the growth and contamination by AFs [5]. Because warm temperatures and humidity provide favorable conditions for fungal growth, AF food contamination is widespread, particularly in tropical regions where temperature and humidity levels are high [11,12]. The carcinogenic, genotoxic, and cytotoxic effects of Aflatoxin B1 (AFB1) are well-documented [13]. The International Agency for Cancer Research categorizes AFs as Class I human carcinogens. In natural systems, all cell membranes mainly consist of polyunsaturated fatty acids. These act as the primary target for AF reactions. Therefore, cellular damage mediated by AFs could relate to free radical liberation through AF metabolism, initiating lipid peroxidation and cell damage [13]. Many investigations have focused on improving food and animal feed by adding medicinal plants or their extracts in various aspects, including reducing the effects of toxins [13,14].

Matrix metalloproteinases (MMPs) are a type of zinc-dependent extracellular matrix (ECM) degrader capable of degrading every component of the ECM. The ECM is a fundamental network involved in embryonic development, angiogenesis, cell repair, and tissue regeneration. MMPs have been associated with several types of cancer, including colon, lung, head and neck, and breast cancer [15]. MMPs are identified by names starting with MMP-1 and ending with MMP-28. They are classified into various categories based on the substrate they attack and their chemical formula and structure. MMP-1 belongs to the interstitial collagenases and degrades types I, II, and III of fibrillar collagens, the main structural components of the ECM. It is postulated to be the reason why collagenases do not participate in the initial stages of carcinogenesis [16,17,18]. MMP-7, also known as elastin, laminin, and fibronectin, is a protein that cleaves collagen, matrilysin, osteopontin, proteoglycans, and entactin. MMP-7 is primarily associated with tissue remodeling in biliary atresia-related liver fibrosis. Increased MMP-7 expression has been observed in various human primary malignancies, including lung, breast, ovarian, and prostate cancer. Both benign and malignant colorectal tumors have been reported to up-regulate MMP-7 [19]. This study aimed to investigate the effect of PG aqueous crude extract on mice orally administered with aflatoxin B1 by monitoring the changes in the expression of Matrix Metalloproteinase, specifically MMP-1 and MMP-7, in the liver, spleen, and kidney using immunohistochemistry (IHC).

MATERIALS AND METHODS

Production, extraction, and quantification of Aflatoxin B1(AFB1)

A pure culture of Aspergillus flavus (A. flavus) was cultivated after isolating A. flavus from 22 Iraqi patients with aspergillosis. A. flavus isolates were microscopically diagnosed before initiating AFB1 production. These isolates were grown on Sabouraud Dextrose Agar medium

(SDA), and subsequently, sterilized rice medium was prepared and distributed in 250 mL Erlenmeyer flasks (25 mL in each flask).

A 5 mm diameter disk was cut from the SDA growth using a sterile cork borer and inserted into the rice medium to create the inoculum. The flasks were then incubated at 28 °C for 21 days [19,20]. After incubation, the moldy rice was soaked for 24 hours in 75 cc of 99.45% chloroform in a shaded room. The soaking medium was then homogenized for 15 minutes using an electric mixer. The extracted solution was sieved using gauze and then passed through Whatman filter paper (grade 1, 110 mm), with the moldy rice deposit rinsed with 50 mL of chloroform before filtering.

The combined chloroform portions were evaporated to dryness at 50 °C. The resultant extract, thickened into a pasty substance, was stored in a refrigerator at 4 °C until use [20].

Purified Aflatoxin B1 (AFB1) Standard

A 10 mg/mL solution of AFB1 was prepared by diluting it in 100% methanol. A 100 µL sample of the dilution was placed in a 2 mL vial, stirred thoroughly, and then further diluted 100,000 times in a mixture of water and methanol (7:3 v/v). The resulting solution was stored in a deep freezer at -70 °C until use [22].

Both thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) were utilized for the quantification and qualification testing of the isolated AFs [21].

According to Park and Troxell [22], a harmful impact dosage of 9 mg/kg body weight (B.W.) of pure aflatoxin B1 extract, reflecting a 90% aflatoxin concentration, was determined based on previous experimental data. For further concentrations, Al-Mudallal, N. H. (2023) [23] utilized 30% and 60% of pure aflatoxin B1, corresponding to 3 mg/kg B.W. and 6 mg/kg B.W., respectively, and confirmed its inductive effect on MMP-1 and MMP-7 expression [24]. Based on the above studies, 60% of pure aflatoxin B1 was used, equivalent to 6 mg/kg body weight.

Preparation of *Panax ginseng* extract and detection of ginsenosides using high-performance liquid chromatography (HPLC)

Powdered roots of Korean ginseng (Panax ginseng) (100 g) were soaked for 8 hours in 700 mL of distilled water (1:7 w/v) and subjected to a water bath at 85–90 ºC with gentle agitation; this step was repeated five times [25,26]. The mixture was then centrifuged at 2000 rpm for 10 minutes. The clear supernatant was filtered through Whatman No.1 paper and concentrated in an oven at 50–55 ºC until it reached a dark brown syrupy consistency. This was then stored in the dark at 4 ºC until use.

Experimental animals

We obtained twelve male Swiss albino mice, ranging in age from 10 to 12 weeks, from the National Center for Drug Control and Research in Baghdad. They were housed in the regulated animal house of Al-Nahrain University in plastic cages, covered with a layer of sawdust, at a temperature of 25 °C, subject to a light/dark cycle of 4-10 hours, and provided with water. The study was approved by the Ethical Committee of Al Iraqi University's College of Medicine in Baghdad, Iraq, with approval No: FM.SA/119.

Experimental design

Animals were exposed to different treatment conditions as suggested by the study carried out by El-Din et al. [27]. The animals were divided into four groups as follows, with three replicates for each group:

Group 1: For two weeks, the animals in the control group were fed pure AFB1 extract (6 mg/kg bw), representing 60% of the concentration twice a week.

Group 2: For two weeks, the animals were given pure AFB1 extract (6 mg/kg bw, at 60% concentration) twice a week and then administered crude ginseng extract (50 mg/kg bw) orally once a day for three weeks.

Group 3: For two weeks, the animals were given pure AFB1 extract (6 mg/kg bw, at 60% concentration) twice a week. They were then administered crude ginseng extract (100 mg/kg bw) orally once a day for three weeks.

Group 4: For two weeks, the animals were given pure AFB1 extract (6 mg/kg bw, at 60% concentration) twice a week. They were then administered crude ginseng extract

(150 mg/kg bw) orally once a day for three weeks.

Preparation of the tissue for the immunohistochemistry

A histopathological study was conducted following the procedures outlined by Bancroft and Gamble [28]. After the animals were sacrificed, various organs (liver, spleen, and kidney) were removed and immersed in a physiological solution (pH 7), prepared according to Benson's method [29]. To attach tissue slices to slides, Mayer's albumin was used. The slides were then placed in a 37 °C oven for 1–2 hours. The washing step was repeated three times, with each wash using 10%, 70%, and 95% concentration solutions. Eosin and Hematoxylin were used to stain the slides, which were left for 10 seconds before being rinsed in water and immersed in 1% concentration acid alcohol. Following these steps, the slides were coated with Distyrene-Plasticizer-Xylene (DPX) and then immersed in Xylene for 15–30 minutes. The histological examination was performed using a light microscope.

Evaluation of matrix metalloproteinases (MMP) 1 and 7 using immunohistochemistry

The damaged liver, kidney, and spleen tissues were embedded in paraffin and incubated in an 80°C hot air incubator for seventy minutes, utilizing positively charged adhesion microscope slides. Subsequently, the slides were rehydrated in graded alcohol. The treated slides were then immersed in xylene for 30 minutes, followed by fresh xylene for an additional 5 minutes. The slides were further processed in 50% ethanol for 5 minutes and distilled water for 5 minutes, allowing for suitable cooling.

For IHC, 40 µL of primary antibodies, including anti-MMP-1 (cat. no. ab52631; Abcam, Cambridge, UK) and anti-MMP-7 (cat. no. ab5706; Abcam, Cambridge, UK), were applied in accordance with the manufacturer's instructions [30, 31].

Evaluation of immunostaining for matrix metalloproteinases (MMP-1 and 7)

Evaluation of immunostaining for matrix metalloproteinases (MMP-1 and 7) MMP-1 and MMP-7 expression were quantified by counting the number of positive cells, identified through cytoplasmic staining with the brown light "3,3'-Diaminobenzidine" stain (DAB) using a 40X light microscope [29, 32]. The evaluation of MMP-1 and MMP-7 immunostaining utilized a scoring system that assessed the extent and intensity of staining in three hotspot areas at an optimal magnification of 40X. Scores ranged from 0 to 100 percent and were classified into weak, moderate, and strong groups [32].

Table 1. Immunostaining expression of the MMP-1 scoring system

MMP-1	Score	Intensity	Stained cells (%)
Negative	0	No staining	<10
Positive	+	Weak	<70
	++	Moderate	>70
	+++	Strong	>80

Table 2. Immunostaining expression of the MMP-7 scoring system

MMP-7	Score	Intensity	Stained cells (%)
Negative	0	No staining	<10
Positive	+	Weak	<40
	++	Moderate	>=40
	+++	Strong	>50

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Packages for Social Sciences (SPSS) version 27. Significance was considered when the p-value was equal to or less than 0.05. The data were presented using simple measurements of means. Additionally, one-way ANOVA was utilized to compare the different organs subjected to variable treatments.

RESULTS

AFB1 production, extraction, and quantification

HPLC investigation of AFs in rice plantation extract showed that the accumulated solvents of acetonitrile: H2O (40:60 v/v) in the system were very suitable after growing A. flavus in rice cultivation extract. AFB1 purification by HPLC and TLC techniques revealed only one peak at an 8.5-minute retention period for AFB1 compared to standard AFs, with a concentration of 0.05 ppm. The rate of retention of every component in the column determines the separation of the mixture into its components.

Estimation of MMP-1 and MMP-7 using immunohistochemistry technique

IHC technique provided definitive data and was presented as counts and percentages. Immunostaining was observed in the AFB1-treated slides as well as in slides treated with aqueous ginseng extract, indicating specificity in the immunohistochemical signals. Immunostaining for MMP-1 and MMP-7 in various mouse tissues was carried out based on the ability of these tissues to absorb DAB cytoplasmic staining. IHC analysis demonstrated positive expression of MMP-1 and MMP-7 in different AFB1-treated tissue organs, as evidenced by brown staining in the cytoplasm of cells, reflecting the effect of DAB pigment (as demonstrated in figures 1, 2, 3, 4, 5, and 6). The stained cells for MMP-1 and MMP-7 expression in 60% AFB1-treated liver sections were counted as 26.12% and 44.33% (number of staining cells according to the DAB and scoring system), respectively. However, their expression in the liver section reduced to 14.00% and 40.03%, respectively, after the addition of 50 mg/kg bw ginseng, compared with the control group. Ginseng extract at 100 mg/kg bw decreased MMP-1 and MMP-7 expression to 10.00% and 37.20%, respectively, when compared to the control group, whereas ginseng extract at 150 mg/kg bw took the upper hand in reducing MMP-1 and MMP-7 expression to 9.76% and 3.30%, respectively, compared to the control group (as listed in table 3).

Table 3. Comparison of MMP-1 and MMP-7 expression in mice liver after being treated with AFB1 and different concentrations of crude PG extract

	Group 1 Control	Group 2	Group 3	Group 4
	60% AFB1	60% AFB1 + (50 mg/kg PG extract)	60% AFB1 + (100 mg/kg PG extract)	60% AFB1 + (150 mg/kg PG extract
MMP-1	26.12	14.00	10.00	9.76
MMP-7	44.33	40.03	37.20	3.30

Figures [1] and [2] illustrate the differences between liver tissues subjected to only AFB1 and those exposed to AFB1 + ginseng crude extract. AFB1-treated liver tissues exhibited a higher degree of MMP-1 and MMP-7 expression ([Figure 1A], [2A]). In contrast, liver tissues treated with AFB1 for 10 days, followed by post-treatment with ginseng crude extract twice a day for two weeks, demonstrated a decrease in MMP-1 and MMP-7 expression levels ([Figure 1B], [2B]).

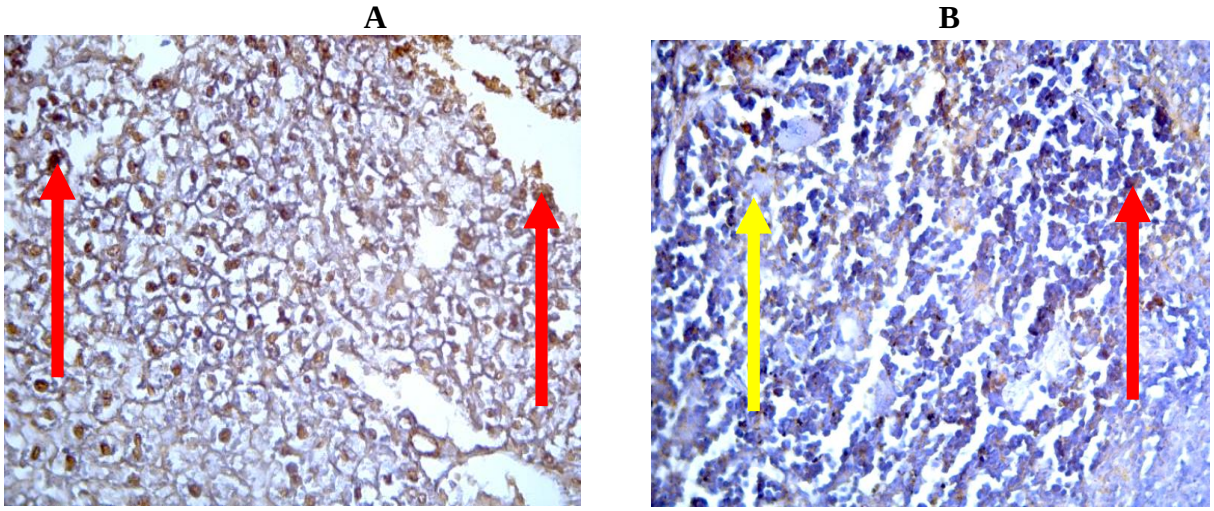

Figure 1. MMP-1 expression in mice liver sections in (A) 60% AFB1treated mice as positive control, (B) AFB1-treated mice liver section then treated with 150 mg/kg bw PG extract (weak expression). Read raw: over expression, yellow raw: downregulated.

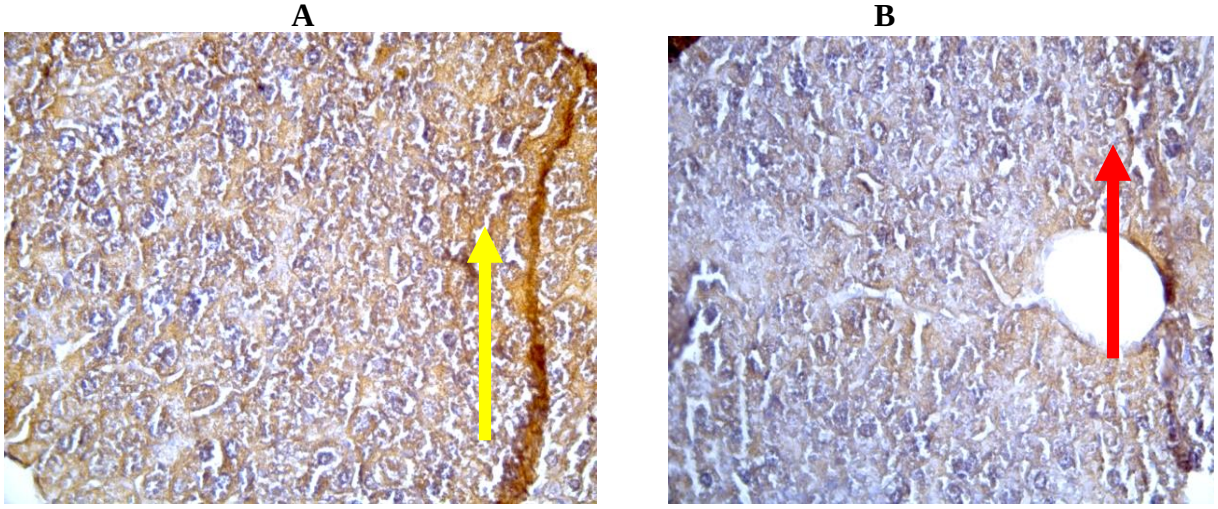

Figure 2. MMP-7 expression in mice liver sections in (A) 60% AFB1 treated mice as positive control, (B) AFB1 + 150 mg/kg bw crude PG extract-treated mice (Moderate expression). Read raw: over expression, yellow raw: downregulated.

A significant reduction in MMP-1 expression was observed in AFB1-administered mice spleen tissues after treatment with different concentrations of crude ginseng extract. In spleen sections, MMP-1 and MMP-7 were expressed at 34.00% and 19.33%, respectively, after treatment with 60% AFB1. The reductions in MMP-1 and MMP-7 expression in group 2 mice were 22.00% and 15.04%, respectively, following treatment with 50 mg/kg bw ginseng, compared to the control group. Ginseng extract at a concentration of 100 mg/kg bw downregulated MMP-1 and MMP-7 expression to 19.12% and 12.20%, respectively, while 150 mg/kg bw of ginseng extract decreased the expression in MMP-1 and MMP-7 to 6.06% and 8.22%, respectively, as compared to the control group (listed in table 4).

Table 4. Comparison of MMP-1 and MMP-7 expression in mice spleen after being treated with AFB1 and different concentrations of crude PG extract

	Group 1Control	Group 2	Group 3	Group 4
	60% AFB1	60% AFB1 + (50 mg/kg PG extract)	60% AFB1 + (100 mg/kg PG extract)	60% AFB1 + (150 mg/kg PG extract)
MMP-1	34.00	22.00	19.12	6.06
MMP-7	19.33	15.04	12.20	8.22

Figure 3 illustrates the differences among spleen sections from tissues subjected to the same treatment by counting the cells that absorbed DAB cytoplasmic staining. Strong MMP-1 expression was observed in the 60% AFB1-treated mice section (as demonstrated in Figure 3A), whereas the highest reduction in MMP-1 expression was demonstrated in spleen tissues treated with 60% AFB1 + 150 mg/kg bw crude extract of ginseng (as shown in Figure 3B).

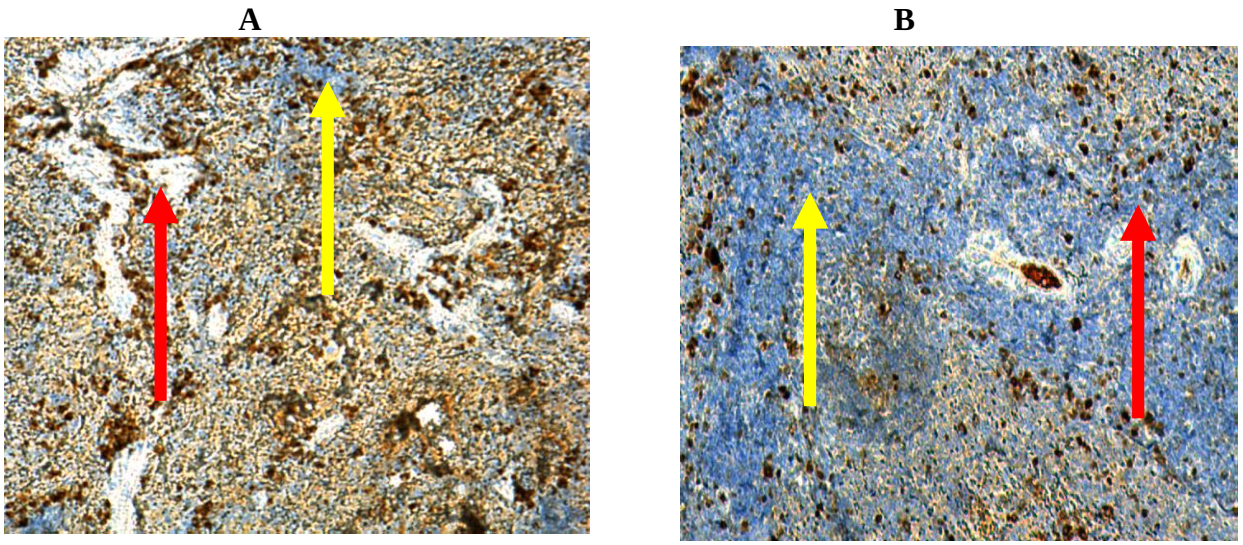

Figure 3. Matrix metalloproteinases 1 (MMP-1) expression in mice spleen section (A) CONTROL MMP-1 expression on 60% AFB1 treated mice (weak expression), (B) spleen section of AFB1 + 150mg/kg bw crude PG extract treated mice (weak expression). Read raw: over expression, yellow raw: downregulated.

In a comparison between MMP-1 and MMP-7 expressions, MMP-7 was expressed as 19.33% after treatment with 60% AFB1, while the expression decreased to 8.22% when treated with 150 mg/kg bw ginseng crude extract (Figure 4).

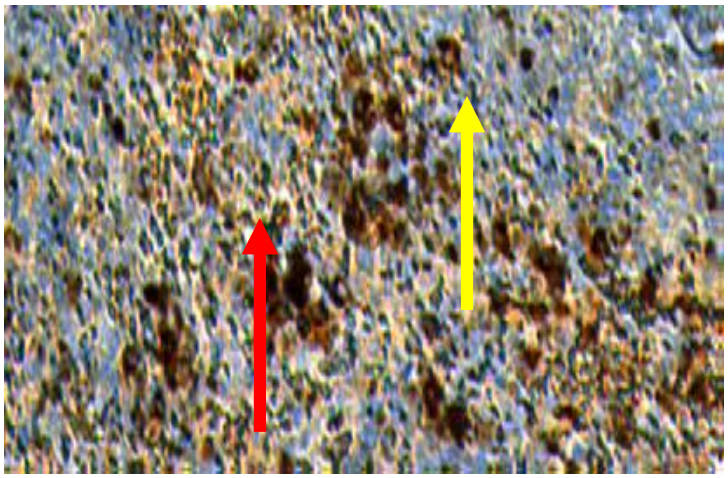

Figure 4. (MMP-7) expression on 60% AFB1-treated mouse spleen (weak expression). Read raw: over expression, yellow raw: downregulated.

In contrast, mice kidney sections exhibited a noticeable downregulation of MMP-1 and MMP-7 expression, with all being graded under weak expression (Table 5).

Table 5. Comparison of MMP-1 and MMP-7 expression in mice kidneys after being treated with AFB1 and different concentrations of crude PG extract.

	Group 1 Control	Group 2	Group 3	Group 4
	60% AFB1	60% AFB1+ (50 mg/kg PG extract)	60% AFB1+ (100 mg/kg PG extract)	60% AFB1 + (150 mg/kg PG extract)
MMP-1	18.00	13.00	7.12	2.23
MMP-7	22.65	9.02	4.23	4.00

Following treatment with 60% AFB1 (Group 1), MMP-1 and MMP-7 expression in kidney tissue appeared to be 18.00% and 22.65% respectively. Post-treatment with 50 mg/kg bw PG aqueous extract (Group 2) resulted in a reduction of MMP-1 and MMP-7 expression to 13.00% and 9.02% respectively.

Further reduction in MMP-1 and MMP-7 expression to 7.12% and 4.23% respectively was observed in Group 3 when mice were post-treated with PG aqueous extract of 100 mg/kg bw. Finally, when PG aqueous extract concentration of 150 mg/kg bw was used for post-treatment in Group 4, a tremendous reduction of MMP-1 and MMP-7 expression was noticed, scoring 2.23% and 4.00% respectively, indicating downregulation compared to control (Group 1) where no post-treatment of PG aqueous extract was applied (Table 5).

Figures 5 and 6 illustrate the immunostaining expression of MMP-1 and MMP-7 in different kidney sections. MMP-1 and MMP-7 were overexpressed when subjected to 60% AFB1, while the expression reduced to weak or negative expression after crude ginseng extract treatment.

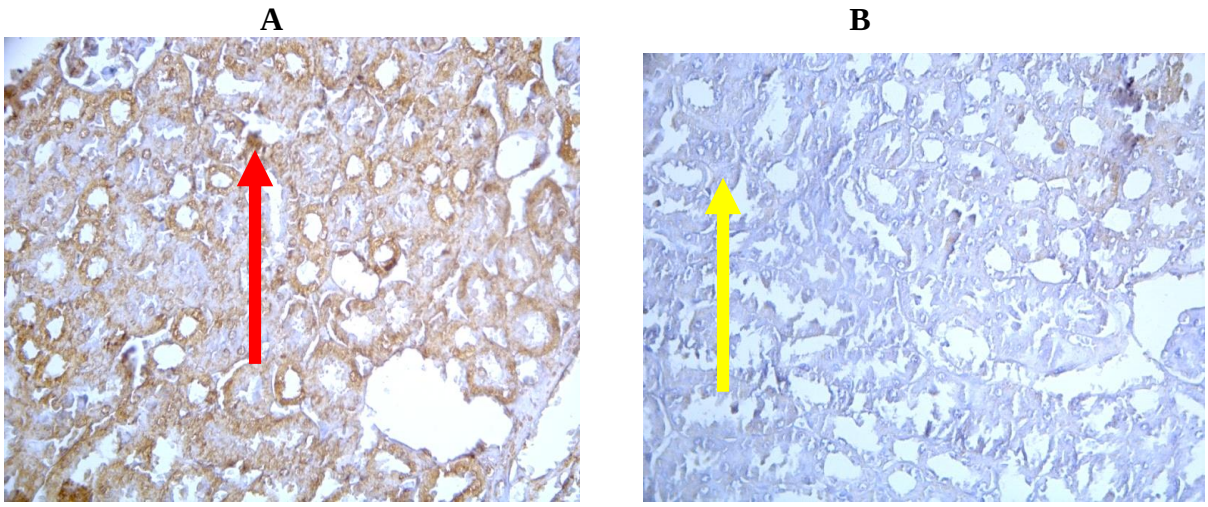

Figure 5: (MMP-1) expression in mice kidney section (A) Control group MMP-1 expression on 60% AFB1 treated mice (strong expression). (B) Kidney section of AFB1 treated mice then post- PG crude treated mice at 150 mg/kg bw (weak expression). Read raw: over expression, yellow raw: downregulated.

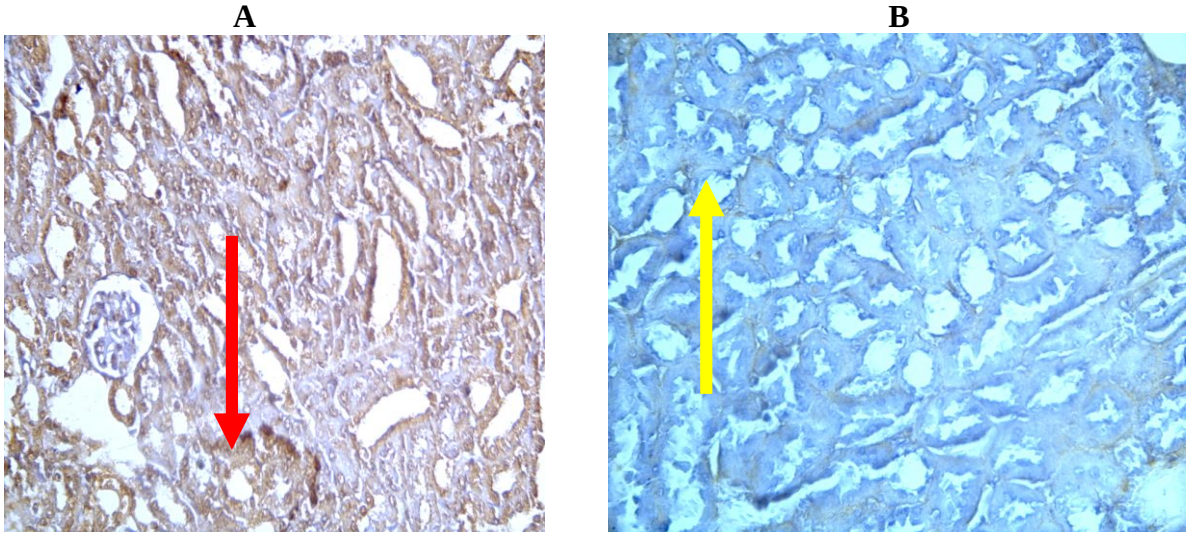

Figure 6. (A) Control: (MMP-7) expression in kidney section of 60% AFB1 treated mice, (B) Kidney section of AFB1-treated mice then post-treated with 150 mg /kg bw crude PG extract (weak expression). Read raw: over expression, yellow raw: downregulated.

For a comparative study of MMP-1 and MMP-7 expression in the above-studied organs, One-Way ANOVA was applied to analyze the relationships between treatment concentrations and marker expression in different organs (Table 6).

Table 6. Comparative study between MMP-1 expression in mice liver, spleen and kidneys after being treated with AFB1 and different concentrations of crude PG extract.

	Control (60%AFB1)	Group 1 60% Aflatoxin B1 + (50 mg/kg PG extract)	Group 2 60% Aflatoxin B1+ (100 mg/kg PG extract)	Group 3 60% Aflatoxin B1 + (150 mg/kg PG extract)
MMP-1 liver	30.36	13.00	14.00	9.69
	24.00	14.00	7.00	10.30
	24.00	15.00	9.00	8.79
MMP-1 Spleen	39.00	22.00	18.24	2.18
	24.00	22.00	18.12	9.00
	39.00	22.00	21.00	7.00
MMP-1 Kidney	18.00	20.00	7.36	1.10
	18.00	10.00	13.00	1.37
	18.00	9.00	1.00	4.10
P		0.294	0.207	0.02*

MMP-1 expression was significantly reduced in the mice liver organ by approximately 3-fold after treatment with a higher experimental dose of PG extract. In contrast, the reduction in effectiveness increased to 9 and 8 folds, respectively, in the spleen and kidney compared with the control. However, the expression reduction with the other PG experimental doses appeared insignificant.

MMP-7 expression was significantly reduced in the mice liver organ by approximately 13 folds, while the reduction in effectiveness decreased to 2.3 and 5.6 folds in the spleen and kidney when treated with a higher dose of PG compared with the control (Table 7).

Table 7. Comparative study between MMP-7 expression in mice liver, spleen and kidneys after being treated with AFB1 and different concentrations of crude PG extract

	Control (60%AFB1)	Group 1 60% AFB1+ (50 mg/kg PG extract)	Group 2 60% AFB1 + (100 mg/kg PG extract)	Group 3 60% AFB1+ (150 mg/kg PG extract)
MMP-7 Liver	45.99	40.00	37.20	5.80
	46.00	40.60	37.20	1.10
	41.00	40.30	37.20	3.00
MMP-7 Spleen	15.99	16.12	12.26	10.63
	14.00	16.00	12.20	10.20
	28.00	13.00	12.20	4.10
MMP-7 Kidney	22.70	7.02	10.00	4.00
	22.79	8.02	1.08	4.00
	22.54	12.02	1.60	4.00
P		0.323	0.088	0.00

DISCUSSION

According to the results obtained from this study, the amount of PG aqueous extract was found to be directly linked to the reduction of AFB1 toxicity. AFB1 metabolism has historically been considered to take place in two stages known as Phase I and Phase II [34]. The CYP450 enzyme systems mediate Phase I metabolism, which consists primarily of enzyme-mediated hydrolysis, reduction, and oxidation reactions that convert AFB1 to more toxic epoxide forms (AFBO). GST enzyme systems mediate Phase II metabolism, involving conjugation reactions of the AFBO intermediates, converting them into more hydrophilic compounds readily extractable in urine or bile [35,36].

In this study, we focused on the effect of AFB1 on the liver and kidneys, as these are the main organs involved in AFB1 metabolism. Additionally, we used the spleen as an example to observe its effect on other body organs. IHC findings confirmed the ability of AFB1 to disrupt the oxidant-antioxidant balance inside affected cells in different body organs at graded levels [37,38].

AFB1 undergoes biotransformation to diverse metabolites that interact with various biomolecules such as nucleic acids, resulting in the formation of AFB-N7-guanine adducts, representing a major DNA damaging event following exposure to AFs. Failure of the repair process leads to alterations in somatic development, especially at the transcriptional level. AFB1 carcinogenicity is mediated by changes in gene expression leading to mutation induction. AFB1 cytotoxicity may be associated with the disruption of cellular membrane integrity by stimulating the lipid peroxidation process in cells [39,40].

Scientifically approved evidence indicates the oxidative and carcinogenic effects of AFB1. Up-regulation of MMPs has been observed in each type of cancer and found to be associated with poor prognosis among cancer patients [41]. MMP-1 overexpression is associated with an increase in hepatocellular carcinoma cells, most likely as a result of ECM degradation during the epithelial-mesenchymal transition (EMT). MMP-7 has also been demonstrated to trigger MMP-1 [42].

Al-Mudallal, N. H. A. L. confirmed that different concentrations of AFB1 and the exposure time were linked to the severity of liver injury and the expression levels of MMP-1 and MMP-7 in the liver [23].

Different types of MMPs are located in the cytosol, subcellular organelles, nucleus, and extracellular regions, serving different roles at different stages, including cell growth, differentiation, survival, and motility. MMPs induced ECM degradation not only support tumor invasion but also alter the behavior of tumor cells, leading to cancer metastasis and ultimately disease progression. Therefore, inhibition of MMPs activity can serve as a useful therapeutic strategy in combating this life-threatening cancer [43,44]. Inhibition of MMPs through downregulation of MMPs expression reduces mRNA expression of MMPs via modulating the Akt signaling pathway [45,46,47].

The crude extract of PG was found to induce potent protective action in laboratory animals and may play a role in the prevention of hepatic, renal, and splenic injury produced by aflatoxins [34]. Several previous studies were carried out on the analysis of PG aqueous crude extract active constituents; PG aqueous extract contained alkaloids, ginsenosides, and another protective component that directly acts as inhibitors of MMPs [48].

Many previous histopathological studies revealed that AFB1 administration induces degenerative changes in the liver, spleen, and kidney.

In this study, we focused on the effect of PG aqueous extract and how it acts against AFB1 toxicity on the liver, spleen, and kidneys. A higher reduction effectiveness was proved in liver tissues after monitoring the expression of MMP-7 in orally 60% AFB1-administered mice, being reduced from 44.33% to 3.30% after treatment with 150mg/kg of PG.

Hashim, S. S. et al. (2016) [48] studied the effect of

PG against AFB1-mediated toxicity on different body organs, and the histological examination of AFB1-treated kidneys showed the appearance of apoptotic cells, degenerative cells, and shrinkage of glomeruli [50]. On the other hand, the kidney sections treated with PG extract appeared to be normal tissue [34].

The hepatotoxic, mutagenic, and carcinogenic effects of AFB1 on several animal models and humans explain the strong efforts of researchers to find any detoxification mechanisms that can be easily applicable [51]. In this study, AFB1 detoxification might be conducted by lowering MMP-1 and MMP-7 marker expression using PG aqueous extract [52], which is an effective strategy against carcinogenesis, mutagenesis, and other kinds of toxicity mediated by carcinogens [49]. The sample size was restricted and considered as one of the limitations in this study.

CONCLUSION

The toxicity reduction effects of PG aqueous crude extract were confirmed against the impact of AFB1 on different living organs involved in AFB1 metabolism. Immunohistochemistry (IHC) analysis indicated a higher reduction effectiveness for both studied markers (MMP-1 and MMP-7), particularly in the expression of MMP-7 in the liver of mice.

REFERENCES

1. Hassan, F.F., Al-Jibouri, M.H., Mustafa, K.A. and Hashim, A.J. Production of Antibody (IgG) Against Aflatoxin B1. *Iraqi J. of Sci*. 2014; 55(2A):394-402.
2. Mushtaq, M., Sultana, B., Anwar, F., Khan, M.Z., and Ashrafuzzaman, M. Occurrence of aflatoxins in selected processed foods from Pakistan. *Int. J. of Mol. Sci*. 2012; 13:8324-8337.
3. Al-Daraji, H.J., AL-Ani, I.A., Minati, J.K., and Al-Hiti, H.EA. Physiological approach to counteract Aflatoxicosis in broiler chickens by the use of Dietary licorice extract. *Iraqi Journal of Agricultural Sciences*. 2005; 36(3):169-178.
4. Shlej, A.A., Saaid, J.M., and Thlij, K.M. The ability of Lactobacillus casei and Lactobacillus acidophilus to protect against toxicity in broiler-fed contaminated diet Aflatoxin B1. *Iraqi Journal of Agricultural Sciences*. 2015; 46(4):652-661.
5. Al-Tekreeti, A. R., Al-Halbosiy, M. M. F., Dheeb, B. I., Hashim, A. J. and Al-Zuhairi, A. F. H. Molecular identification of clinical Candida isolates by simple and randomly amplified polymorphic DNA-PCR. *Arab J Sci Eng*. 2017. DOI 10.1007/s13369-017-2762-1**.**
6. Diarra, Q., Babana, A.H., and Maïga, K. Fungal. Profile and Mycotoxin Contamination in Animal Feed in Urban and Peri-urban Zones of Bamako. *Int. J. Curr. Res. Biosci. Plant biol*. 2017; 4(11):50-56.
7. Dheeb, B. I., Mohammad, F. I., Hadi, Y. A., and Abdulhameed, B. A. Cytotoxic Effect of Aflatoxin B1, gliotoxin, Fumonisin B1, and Zearalenone Mycotoxins on HepG2 Cell Line in vitro. *Int. J. Adv. Res.* 2013; 1(8):355-363.
8. Abdulateef S.M. and Al-Judy N. J. Sulforaphane (SFN) and bardoxolone (CDDO)-induced inhibition of aflatoxinb1-mediated genotoxicity in human lymphocytes: Role of Gstm1, Gst1a1 and Nrf2 gene expression. *Journal of Pharmaceutical Science & Research*. 2019; 11(1):216-220.
9. Hussein, H.S., Dheeb B.I., Hamada, T.A. Studying the candida resistance and sensitivity for some antifungals. *Journal of Biotechnology Research Center*.2019; (2)25-34.
10. Pietruszewska, W., Bojanowska-Poźniak, K. and Kobos, J. Matrix metalloproteinases MMP-1, MMP-2, MMP-9, and their tissue inhibitors TIMP1, TIMP2, TIMP3 in head and neck cancer: an immunohistochemical study. *Otolaryngol Pol.* 2016; 70(3):32-43,

11. Al-Daraji, H.J., AL-Ani, I.A., Minati, J.K., and Al-Hiti, H.E. Use of Licorice Extract in counteracting Aflatoxicosis in Broilers. *Iraqi Journal of Agricultural Sciences*. 2005; 36(2):197-206.
12. Mustafa, M.M., Karadas, and Tayeb, I.T. Adding different levels of Turmeric powder and Curcumin in the diet on some serum biochemical of Broiler reared under normal and heat stress conditions. *Iraqi Journal of Agricultural Sciences*. 2021; 52(1):10-19.
13. Dheeb, B. I. Immunohistochemical study of Tumor Necrosis Factor-alpha (TNF- α) expression in lung, liver, and spleen during aspergillosis infection. *BMC genomics*. 2014; 15(2):71.
14. Reem M. Azeez, Wisam K. AL-Hashemi, Tawfeeq F. R. Al-Auqbi and Jamel Jebali. Relation of MDA as Oxidative Stress Marker with Lipid Profile in a Diabetic Patient. *Al-Nahrain Journal of Science ANJS*. 2023; 26 (3):12-17
15. Dahham, M.T., Omar, A.F., Dheeb B.I. Synergistic effect of tea tree oil on fungi causing vaginal thrush in pregnant women. *Journal of Biotechnology Research Center 13*. 2019; (2)35-44.
16. Dheeb, B. I., Al-Mudallal, N. H., Salman, Z. A. and Ali, M. The Inhibitory Effects of Human, Camel, and Cow's Milk against Some Pathogenic Fungi in Iraq. *Jordan Journal of Biological Sciences*. 2015; 8(2):89-93.
17. Babu, G. R., Prasad, M. G., and Prasad, T. N. V. K. V. Isolation and quantification of Aflatoxin from *Aspergillus flavus* infected rice. *Int. J. Pure Appl. Sci. Technol*. 2011; 5(1):16-24.
18. Kosalec, I., Pepeljnjak, S., and Jandrlic, M. Influence of media and temperature on gliotoxin production in Aspergillus fumigatus strains. Arhivza Higijenu Rada I toksikologiju. *Archives of industrial hygiene and toxicology*. 2005; 56(3):269-273.
19. Rassin, N. K., Nemat J. A., Dheeb, B. I. Molecular Identification of Aspergillus fumigatus Using ISSR and RAPD Markers. *Iraqi Journal of Science*. 2015; 56(4A), 2788-2797.
20. Juber, K.S. and AL-Runaiee, H.A. Detection of the fungus and its toxins associated with cotton seeds and cakes and evaluation of their effects on seed germination. *Iraqi journal of Agricultural Sciences*. 2008; 39(4):1-14.
21. Hussain, A.F., Sulaiman, G.M., Dheeb, B.I., Hashim, A.J., Histopathological changes and expression of transforming growth factor beta (TGF-β3) in mice exposed to gliotoxin. *Journal of K S U – Science*. 2018; 27:193–197.
22. Park D. and Troxell T. Mycotoxins and food safety. *Adv Exp Med Biol*. 2002; 504:173-9.
23. Al-Mudallal, N. H. A. L. The Expression of MMP-1 and MMP-7 in mice liver after exposure to aflatoxin B1 using immunohistochemistry technique. *Archives of Razi Institute*. 2023; 78(1):63-72.
24. Dheeb, B.I., Al-dujayli, S.M.A., Ibrahim, I.M., Abbas, Q.A. Study the Antifungal Activity of ZnS: Mn Nanoparticles Against Some Isolated Pathogenic Fungi. *Journal of Physics: Conference Series.* 2019; 1178:46–52.
25. Corbit, R., Ebbs, S., King, M. L., and Murphy, L. L. The influence of lead and arsenite on the inhibition of human breast cancer MCF-7 cell proliferation by American ginseng root (*Panax quinquefolius* L.). *Life sciences*. 2006; 78(12):1336-1340.
26. Hashim S. S, Mahmood Z. F, Abdulateef S. F, Dheeb B. I. Evaluation Cytotoxicity Effects of Centaurea Cineraria Extracts Against some of Cancer Cell Lines. *Biomed Pharmacol J*. 2023; 16(1).
27. El-Din, M. M. M., Mostafa, A. M. and Abd-Elkader, A. Experimental studies on the effect of Lambda-Cyhalothrin insecticide on lungs and the ameliorating effect of plant extracts Ginseng (Panax Ginseng) and garlic (Allium sativum L.) on asthma development in albino rats. *BMC research notes*. 2014, 7(1):243.
28. Bancroft JD. and Gamble M. Theory and practice of histological techniques: Elsevier health sciences. 2008.
29. Benson. Microbial Applications Laboratory Manual in General Microbiology: The McGraw-Hill Companies; 2001.

30. Dheeb, B.I., Hashim, S.S., Hussein, H.S., Hamada, T.A. Study of TGF- β Immune Marker Expression in Mice Exposed to Candida Spp. *AIP Conference Proceedings*. 2022.
31. Mahmood, M.N., Mahal, M.H., Mohammed, H.E., Tariq, T.A., Mohammed, A.J. Dheeb. B.I. Evaluation of enzymes liver and kidney function in serum people the exposures at risk of chemicals volatile in the lab pharmaceutical samarra. *Journal of Global Pharma Technology*. 2019, 11(7):331–336
32. Wu, X. G., Zhu, D. H., and Li, X. Anticarcinogenic effect of red ginseng on the development of liver cancer induced by diethylnitrosamine in rats. *J. of Korean med. Sci*. 2001; 16(SUPP):S61-S65.
33. Griselda A Cabral-Pacheco, Idalia Garza-Veloz, Claudia Castruita-De la Rosa, Jesús M Ramirez-Acuña, Braulio A Perez-Romero, Jesús F Guerrero-Rodriguez, Nadia Martinez-Avila, and Margarita L Martinez-Fierro. The Roles of Matrix Metalloproteinases and Their Inhibitors in Human Diseases. *International Journal of molecular science*. 2020. Doi: 10.3390/ijms21249739
34. Whittaker, M., Floyd, C.D., Brown, P., Gearing, A.J. Design and therapeutic application of matrix metalloproteinase inhibitors. *Chem. Rev*. 1999; 99,2735–2776.
35. Juni Jekti Nugroho, Andi Sumidarti, Mufliha Siri, Husni Cangara, Nurhayaty Natsir, Maria Tanumihardja, Noor Hikmah, Asrianti1 Matrix. Metalloproteinase-1 (MMP-1) Expression and Density of Collagen Fibers Following Application of Haruan Fish (*Channa striata*) extract in Inflamed Pulp of Wistar Rat. *Sys Rev Pharm*. 2020; 11(9):6-9.
36. Abdulbaqi, N. J. and Dheeb, B. I. and Irshad, R R. Expression of Biotransformation and Antioxidant Genes in the Liver of Albino Mice after Exposure to Aflatoxin B1 and an Antioxidant Sourced from Turmeric (Curcuma longa). *Jordan Journal of Biological Sciences*. 2018; 11(2):89-93.
37. Abcam. ab80436 – EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC Kit Version 5 Last updated 3 November .2017.
38. Kim S. J., Pham T. H., Bak Y., Ryu H. W., Oh S. R., Yoon Y. D. 7-Methoxy-luteolin-8-C-β-6-deoxy-xylo-pyranos-3-uloside exactly (mLU8C-PU) isolated from Arthraxon hispidus inhibits migratory and invasive responses mediated via downregulation of MMP-9 and IL-8 expression in MCF-7 breast cancer cells. Environ. 2018 Toxicol. 33, 1143–1152. 10.1002/tox.22620.
39. Abdullah, H.I., Hammadi, S.Y., Hussein, A.S., Dheeb, B.I. Investigation of genetic diversity and relationships among the clinical candida species using random amplified polymorphic DNA (RAPD) analysis. *Research Journal of Biotechnology*. 2019; 14(Special Issue 1):6–13.
40. Awad, F.M., Al-Samarrai, A.H.M., Dheeb, B.I. Study of the inhibitory effects of rheum ribes extracts on a pathogenic fungi and cancer cell line. *Plant Archives*. 2020; 20(1):3161–3168.
41. Abdulateef, S.M. Cytotoxicity and Genotoxicity of AflatoxinB1 on Human Lymphocytes In vitro. A Thesis Submitted to the College of Science/ University of Baghdad in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy (Ph.D.) in Biology/Botany. 2019.
42. Bbosa, G.S., Kitya, D., Odda, J. and Ogwal-Okeng, J. Aflatoxins metabolism, effects on epigenetic mechanisms and their role in carcinogenesis. 2013; 5(10):14.
43. Cabral-Pacheco GA., Garza-Veloz I., Castruita-De la Rosa C., Ramirez-Acuna JM., Perez-Romero BA., Guerrero-Rodriguez JF., et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. *Int J Mol Sci*. 2020; 21(24):9739.
44. Kennedy, D. O. and Scholey, A. B. Ginseng: potential for the enhancement of cognitive performance and mood. *Pharmacology Biochemistry and Behavior*. 2003; 75(3): 687-700.
45. Blumenthal, M. (2003). The ABC clinical guide to herbs. American Botanical Council.
46. Speijers, G.J.A. and Speijers, M.H.M. Combined toxic effects of mycotoxins. *Toxicology Letters*, 2004; 153:91-98.

47. Lai Y-L., Gong C-L., Fu C-K., Yueh T-C., Tsai C-W., Chang W-S., et al. The contribution of matrix metalloproteinase-1 genotypes to hepatocellular carcinoma susceptibility in Taiwan. *Cancer Genom Proteom*. 2017; 14(2):119-25.
48. Hashim, S. S. and Shawkat, M. S. Protective and Creative Effects of Panax ginseng Aqueous Crude Extract in Histopathological Changes of BALB/c Mice Exposed to Aflatoxins. *Egyptian Acad. J. of Biol. Sci., D. Histology & Histochemistry*. 2016; 8(1):15-24.
49. Duffy M. J., Maguire T. M., Hill A., McDermott E., O'Higgins N. Metalloproteinases: role in breast carcinogenesis, invasion and metastasis. *Breast Cancer Res*. 2001; 2:1–6.
50. Dheeb, B. I., Abdulla, S. N., Shanter ALQaysi, S. A., Al-Sarraj, B. M., & Farhan, M. S. Extraction of Klebsiella pneumoniae and Candida albicans Biofilm and Studying their Cytotoxic Effects on Human Lymphocytes. *Jordan Journal of Biological Sciences*. 2023; 16(4).
51. Mohammed, G. A. Studying the Anti Candidal-Activity of Different Herbal Oils Incorporated into Tissue Conditioner:(A Comparative study). *Jordan j. pharm. sci*. 2023; 16(4):871-879.
52. Alholy, T. and Khaddam, W. Extracellular Synthesis of Magnesium Oxide at Nano and Bulk Scale: Antifungal Effect Against Candida albicans, Aspergillus Niger. *Jordan j. pharm. sci*. 2023; 16(4):740-752.

تأثر تعبير MMP-1 وMMP-7 بالجينسينوسيدات في الفئران المعرضة للأفلاتوكسين: B1 في دراسة على الجسم الحي

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ملخص

تعد الجينسينغ الباناكس (PG) واحدة من الأدوية العشبية الأكثر استخدامًا بشكل عام، وقد أظهرت أن لديها مجموعة متنوعة من التأثيرات الإيجابية مثل التأثير المضاد للالتهابات والمضاد للأكسدة ومضاد للسرطان. يعمل الجينسينويدات الطبيعية الموجودة في نبات الجينسنغ على تثبيط انتشار الخلايا وتقليل بوضوح الضرر الكبدي الناجم عن بعض المواد الكيميائية. يعد الأفلاتوكسين ب1 (AFB1)واحدة من أبرز السموم الفطرية، نظرًا لتأثيراتها الضارة على الكبد والمناعة وتسببها في تكون الأورام في النماذج الحيوانية والبشر. في هذه الدراسة، تم دراسة تأثيرات مستخلص الجينسنغ الخام بتراكيز مختلفة على تعبير المصفوفة المعدنية البروتينية 1 و 7 (MMP-1) و (MMP-7) في الكلى والطحال والكبد لفئران تعرضت تجريبيًا لمادة الأفلاتوكسين ب1 باستخدام تقنية التصبيغ المناعي الكيميائي للانسجة (IHC) ، أُعطيت الفئران 6 ملغ/كغ من وزن الجسم (bw) من الأفلاتوكسين ب1 المنقى المعزول والمستخلص من فطر الاسبرجلاس فلافس *Aspergillus flavus* ، تركيز 0.05 جزء في المليون عن طريق الفم مرتين في الأسبوع لمدة أسبوعين. ثم تمت مقارنة تأثيرات ثلاث جرعات مختلفة (50 , 100, 150) ملغ/كغ من وزن الجسم من الجينسنغ الخام. تم تقدير تعبير المصفوفة المعدنية البروتينية MMP-1وMMP-7 في الأعضاء باستخدام تقنية IHC، تم استخدام 6 ملغ/كغ من الأفلاتوكسين ب1 المنقى، الذي يمثل تركيزًا بنسبة 60٪، كمجموعة سيطرة. أظهرت تحليل IHC أن التعبير عن المصفوفة المعدنية البروتينية MMP (1,7) في الطحال والكبد والكلية للفئران قد قل عقب العلاج بمستخلص الجينسنغ الخام. انخفض تعبير MMP-1 في الكبد بنسبة تقريبية 2.6 مرة، في حين بلغ الانخفاض الفعال ل MMP-1 إلى 9 و8 مرات على التوالي في الطحال والكلية عند معالجتها بجرعة أعلى من الجينسنغ مقارنة بالسيطرة. انخفض تعبير MMP-7في الكبد بنسبة تقريبية 13 مرة، في حين انخفض التعبير الفعال ل MMP-7 إلى 2.3 و5.6 مرات في الطحال والكلية عند معالجتها بجرعة أعلى من الجينسنغ مقارنة بالسيطرة. لوحظ تقليل تعبير MMPs بفعالية بفعل مستخلص الجينسنغ الخام في مواجهة تأثير الأفلاتوكسين ب1 على الأعضاء الحية المشاركة في أيض الأفلاتوكسين ب1 ،أشار تحليل IHCإلى وجود تقليل اكثر فعالية في تعبير MMP-7في مقارنة بين كلا المؤشرين المدروسين في كبد الفئران.

الكلمات الدالة: الجينسنغ، الأفلاتوكسين ب1، المصفوفة المعدنية البروتينية.

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المجلة الأردنية في العلوم الصيدلانية

تعريف بالمجلة الأردنية في العلوم الصيدلانية

تأسست المجلة الأردنية في العلوم الصيدلانية بقرار لجنة البحث العلمي/ وزارة التعليم العالي والبحث العلمي رقم 367/2/10 تاريخ 2007/1/11 بشأن إصدار "**المجلة الأردنية في العلوم الصيدلانية**" ضمن إصدارات المجلات الأردنية الوطنية، وهي مجلة علمية عالمية متخصصة ومحكمة، وتصدر بدعم من صندوق دعم البحث العلمي والجامعة الأردنية تعنى بنشر البحوث العلمية الأصيلة المقدمة إليها للنشر في كافة مجالات العلوم الصيدلانية والعلوم الأخرى المرتبطة بها. وتصدر عن عمادة البحث العلمي وضمان الجودة في الجامعة الأردنية باسم الجامعات الأردنية كافة، خدمة للمتخصصين والباحثين والمهتمين في هذه المجالات من داخل الأردن وخارجه. وهي مجلة تصدر أربع مرات في العام أعتبارا من 2021، ومواعيد صدورها (آذار وحزيران وأيلول وكانون أول) من كل عام.

وباسمي وباسم أعضاء هيئة التحرير نود أن نشكر الزملاء الذين أسهموا بإرسال أبحاثهم إلى مجلتنا وتمكنا من إخراج العدد الأول. ونأمل من جميع الزملاء بإرسال ملاحظاتهم الإيجابية إلينا لنتمكن من النهوض بمجلتكم بالشكل الذي يليق بها.

وهذه دعوة إلى كافة الزملاء لإرسال اسهاماتهم العلمية من الأبحاث الأصيلة إلى عنوان المجلة.

والله ولي التوفيق

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