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Aims & Scopes:

***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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Distribution of Bacteria according to Drug Resistance among Adult Women with Bacteriuria in Samara City

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ABSTRACT

Background: Bacteriuria is defined as the presence of bacteria in urine without the accompanying signs and symptoms of a urinary tract infection. The most common bacterium causing bacteriuria is E. coli. About 1-5% of healthy premenopausal women and 1.9-9.5% of pregnant women have bacteriuria. The most effective drugs based on urine culture results were Nitrofurantoin (98.3%), followed by Cefuroxime (89.3%) and Cotrimoxazole (20%).

Aim and objectives: To identify the distribution of bacteria according to drug resistance among adult women with bacteriuria in Samara city, Iraq, and to determine certain influencing factors.

Materials and Methods: This descriptive cross-sectional study was conducted on adult women (18-44 years) attending Samara General Hospital. Demographic information and investigation results were obtained and reported using an appropriate questionnaire. A patient with asymptomatic bacteriuria was identified when one species of bacteria grew in the urine with at least 100,000 colony-forming units (CFUs) per milliliter, regardless of the presence of pyuria, even in the absence of any UTI symptoms. Frequencies (number of cases) and percentages were used to statistically describe the data where appropriate. Comparison between the study groups was performed using the Chi-square ($\chi 2$) test. P values less than 0.05 were considered statistically significant.

Results: The frequency of bacterial growth in the sample was 19%. The highest frequency of cases was among the age group 28-37 years (22.4%). Positive cases were more prevalent among pregnant women (21.4%) than non-pregnant women (13.3%). Staphylococcus was the most frequently identified bacterium (42%), followed by E. coli (39%), Klebsiella (11%), and Streptococcus (8%). The drug with the highest sensitivity to bacterial growth was Amikacin, followed by Meropenem.

Conclusions: The frequency of bacterial growth was 19%. The most frequently isolated bacteria from the culture were Staphylococcus, followed by E. coli. The most sensitive drug was Amikacin, followed by Meropenem.

Keywords: Bacteriuria, drug resistance, Samara.

INTRODUCTION:

Bacteriuria is the presence of bacteria in urine without any accompanying symptoms of a urinary tract infection (UTI) (1-3). Age-related bacteriuria is common in adult women (18 years and older) (4,5). Escherichia coli continues to be the most frequently cultivated organism in both community-dwelling and institutionalized individuals, despite some variances in the incidence of common urinary tract pathogens (6). Almost all research on the bacteriologic criteria used to diagnose UTIs has been conducted in populations that are primarily female(7).

E. coli represents 60-90% of infections in women (1,8,9-11). Other bacteria include Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, and group B

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Streptococci (3,12,13). Gram-positive organisms like Staphylococcus saprophyticus also cause bacteriuria (14). Certain studies have documented that E. coli is the most common bacterium (65.5%), followed by Klebsiella (20.7%) (15), while another study found E. coli represented 57%, followed by Staphylococcus aureus (22.5%) (16). In another study, the most common bacterium was S. aureus (31.2%), followed by E. coli (25%) (17). Up to 10% of women may have a urinary tract infection in a given year (18). About 1-5% of healthy premenopausal women (19) and 1.9-9.5% of pregnant women (9,20) have bacteriuria.

The reduction in effectiveness of a medication, such as an antimicrobial or an antineoplastic, in treating a disease or condition is called drug resistance (21). Medicines used in the prevention and treatment of infections in humans, animals, and plants are called antimicrobials. These include antibiotics, antivirals, antifungals, and antiparasitics. Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi, and parasites change over time and no longer respond to medicines (22).

Using drug sensitivity tests to determine the types of bacteria and the most suitable antibiotics to be used, as revealed in urine culture and urine analysis, is essential (23,24). A study conducted in Uganda revealed that the most effective drugs for treating bacteria causing bacteriuria among adult nonpregnant women, based on urine culture results, were Nitrofurantoin (98.3%), followed by Cefuroxime (89.3%) and Cotrimoxazole (20%) (16).

Personal knowledge about antibiotic use and resistance is crucial in the treatment of bacterial infections. A study conducted among Al-Yarmouk University students (both undergraduate and postgraduate) revealed that about 72.7% of the sample had knowledge about antibiotic use and resistance (25). Another study in Jordan revealed that 65% of the sample had heard about the term "antibiotic resistance" from medical staff (26).

MATERIALS AND METHODS:

This study was conducted at Samara General Hospital from July 2022 to March 2023. The hospital is located in Samara city, which is about 120 km north of Baghdad. The study sample consisted of adult women attending outpatient clinics at Samara General Hospital who did not present signs and symptoms of UTIs.

Study design: A cross-sectional study was conducted among adult women (18-44 years) attending Samara General Hospital outpatient clinics. Consent was obtained from all participants.

Study population: The study included all adult women aged 18-44 years attending Samara General Hospital outpatient clinics during the study period who fulfilled the inclusion criteria. The sample size was 500 adult women.

Inclusion criteria: All adult women aged 18-44 years who attended outpatient clinics at Samara General Hospital during the study period, women with asymptomatic bacteriuria, and those willing to participate in the study were included.

Exclusion criteria: Women with a history of antibiotic therapy in the previous 2 weeks and those with serious or chronic diseases were excluded.

Bacterial culture, identification, and antimicrobial susceptibility testing: After obtaining consent, demographic information was collected from the women using a predesigned questionnaire. About 5 mL of freshly voided midstream urine samples were collected using a sterile screw-capped, wide-mouth container. The women cleansed their genitals with clean water and collected the midstream urine into the wide-mouthed container.

After collecting midstream urine samples from the women in sterile bottles, the samples were examined in the laboratory within 3 hours. The urine samples were inoculated on Cystine Lactose Electrolyte Deficient (CLED) agar and incubated at 37°C for 24 hours (27). Blood agar and MacConkey agar media were used for cultures and subcultures. The appearance of 100,000 Colony Forming Units (CFU) per milliliter on blood agar and MacConkey agar was considered positive (6,7,27-30). The identification of bacteria depended on their gram staining, cultural

morphology, and biochemical characteristics (28).

Bacterial isolates were identified using colony features, Gram-staining, and a variety of biochemical assays (such as Kligler Iron Agar (KIA), Sulphur Indole Motility (SIM) media, citrate, oxidase, urease, catalase, and coagulase) in accordance with conventional bacteriological protocol. The antimicrobial susceptibility test was conducted using Muller-Hinton agar medium and the Kirby-Bauer disk diffusion technique. The diameter of the zone of inhibition was evaluated in accordance with the Clinical Laboratory Standard Institute (CLSI) 2017 guidelines (30). For statistical analysis, the Chi-square test was used. A P-value of less than or equal to 0.05 was considered significant.

RESULTS:

According to Table 1, the sample group had a frequency of asymptomatic bacteriuria of 19%. The highest frequency of cases was observed among the age group of 28-37 years (22.4%), followed by the age group younger than 28 years (14.3%), showing a significant difference. The frequency of cases was slightly higher among those with secondary education or less (20%) compared to those with higher than secondary education (18.2%), but without a significant difference. There was a higher prevalence of cases among pregnant women (21.4%) compared to non-pregnant women (13.3%), indicating a significant difference between the two groups.

Figure 1 reveals that Staphylococcus was the most frequently isolated bacteria (42%), followed by E. coli (39%), Klebsiella (11%), and Streptococcus (8%).

Table 2 shows that among pregnant women, the most frequent bacteria isolated from urine cultures were E. coli (45.3%), followed by Staphylococcus (40%), Klebsiella (10.7%), and Streptococcus (4%). Among non-pregnant women, the frequencies were Staphylococcus (50%), Streptococcus (25%), E. coli (15%), and Klebsiella (10%).

Figure 2 shows that Staphylococcus bacteria were sensitive to the following drugs: Amikacin (94%), Ciprofloxacin (82%), Vancomycin (79%), Meropenem (79%), and Ofloxacin (61%). E. coli showed sensitivity to Amikacin (88%), Ciprofloxacin (88%), Vancomycin (87%), Meropenem (63%), and Ofloxacin (50%). Streptococcus bacteria exhibited sensitivity to Amikacin (92%), Ciprofloxacin (77%), Vancomycin (92%), Meropenem (92%), and Ofloxacin (92%). Klebsiella bacteria were sensitive to Amikacin (100%), Ciprofloxacin (50%), Vancomycin (0%), Meropenem (100%), and Ofloxacin (0%). Overall, the most effective drug against bacterial growth was Amikacin, followed by Meropenem.

DISCUSSION:

In the current study, the frequency of bacteriuria among the sample group was 19%. This result was higher than that found in certain studies (6-10%) (31,32), 13% (33), 17% (16), and 12% (8). The current result was lower than that found in other studies (81%) (34) and (60%) (35). This difference may be attributed to variations in vaginal pH among women (36).

Regarding age groups, it was found that the highest frequency of cases was among the age group 28-37 years (22.4%), followed by the age group younger than 28 years (14.3%), and the lowest among the age group older than 37 years (11.5%). Other studies reported that the most frequent cases were among the age group 18-27 years (45.3%), followed by the age group 28-37 years (25.26%), and the lowest among the age group 38 years and older (10.42%) (34). Meanwhile, other studies revealed that the highest frequency was among the age group 25-30 years (62.5%) (17,15), and another found that the highest frequency was among the age group 15-24 years (37); in Cameroon, the highest frequency was among the age group 20-39 years (38). This difference may be attributed to the sexual activity of women in these age groups, which is considered a risk factor (39).

Table (1) Distribution of cases according to certain demographic features.

Personal character \ Bacterial urine culture		Yes	No	Total	Chi square test
Age group (years	Less than 28	5 (14.3%)	30 (85.7%)	35 (100%)	The chi-square statistic is 7.707. The *p*-value is .021206. The result is significant at $p < .05$.
	28-37	75 (22.4%)	260 (77.6%)	335 (100%)	
	more than 37 years	15 (11.5%)	115 (88.5%)	130 (100%)	
	Total	95 (19%)	405 (81%)	500 (100%)	
Education	Secondary and less	45 (20%)	180 (80%)	225 (100%)	The chi-square statistic is 0.2658. The *p*-value is .606152. The result is *not* significant at $p < .05$.
	More than secondary	50 (18.2%)	225 (81.8%)	275 (100%)	
	Total	95 (19%)	405 (81%)	500 (100%)	
Pregnancy	Yes	75 (21.4%)	275 (78.6%)	350 (100%)	The chi-square statistic is 4.4711. The *p*-value is .034474. The result is significant at $p < .05$.
	No	20 (13.3%)	130 (86.7%)	150 (100%)	
	Total	95 (19%)	405 (81%)	500 (100%)	

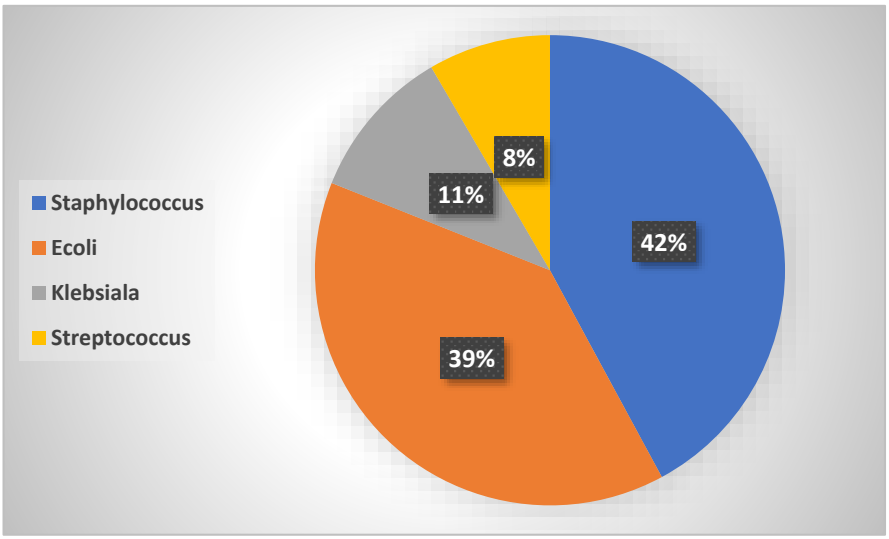

Figure (1) Distribution of cases according to types of bacteria isolation

Table (2) Distribution of cases according to presence of pregnancy and type of bacteria.

Pregnancy presence / Bacteria type	Pregnant		Nonpregnant		Total	
Staphylococcus	30	(40%)	10	(50%)	40	(42.1%)
E. coli	34	(45.3%)	3	(15%)	37	(38.9%)
Klebsiella	8	(10.7%)	2	(10%)	10	(10.5%)
Streptococcus	3	(4%)	5	(25%)	8	(8.4%)
Total	75	(100%)	20	(100%)	95	(100%)

The chi-square statistic is 12.3806. The *p*-value is .006187. The result is significant at $p < .05$.

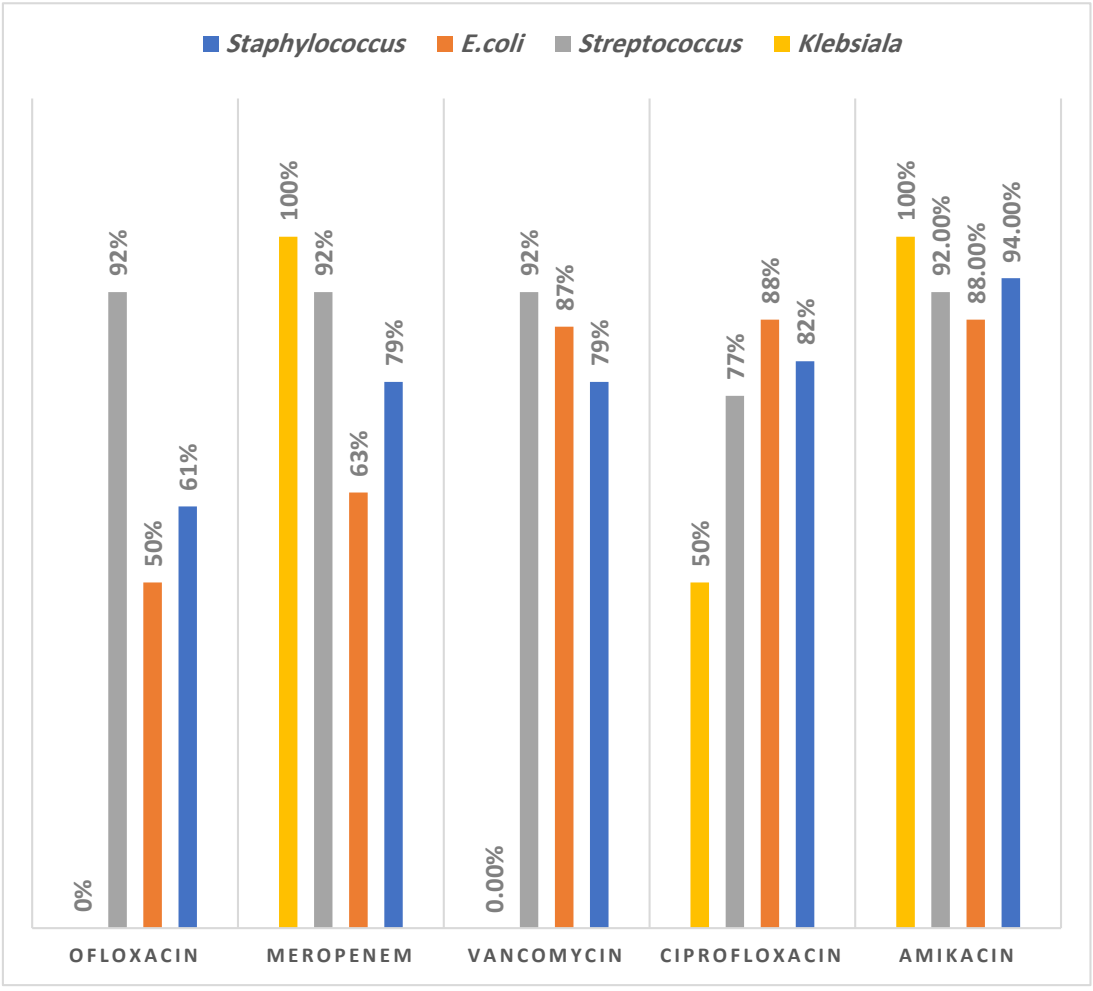

Figure (2) Distribution of bacteria type according to sensitivity to certain antibiotics

Regarding educational level, there were no significant differences similar to those reported by other studies (17,40).

The most frequent bacterial cause of bacteriuria among the sample cases was Staphylococcus (42%), followed by E. coli (39%), Klebsiella (11%), and lastly Streptococcus (8%). Other studies also revealed that the highest frequency of causative bacteria was Staphylococcus followed by E. coli (17,41), while others reported that E. coli had the highest frequency (15,16,34,37,42,43,44).

Among pregnant women, the most frequent bacteria was E. coli (45.3%), and among non-pregnant women, it was Staphylococcus (50%). Other studies found that the highest frequency of causative agents was E. coli among both pregnant women and controls (8).

Regarding pregnancy status, the frequency of bacteriuria cases was more frequent among pregnant women (21.4%) compared to non-pregnant women (13.3%), with a significant difference. These results are consistent with another study reporting that positive cultures were more frequent among pregnant women (14%) than among non-pregnant women (12%) (8).

Staphylococcus showed sensitivity to the following drugs: Amikacin (94%), Ciprofloxacin (82%), Vancomycin and Meropenem (79%), and Ofloxacin (61%). In another study, it was found that Staphylococcus bacteria were sensitive to cefuroxime, cephalexin, Amikacin, and gentamicin (34). E. coli exhibited sensitivity to the following drugs: Amikacin and Ciprofloxacin (88%), Vancomycin (87%), Meropenem (63%), and Ofloxacin (50%). Another study found that E. coli was sensitive to Cefuroxime, Nitrofurantoin, Cephalexin, Amikacin, and Gentamicin (34).

In the current study, Klebsiella bacteria showed sensitivity to the following drugs: Amikacin and Meropenem (100%), Ciprofloxacin (50%), and Vancomycin and Ofloxacin (0%). Another study revealed that this bacterium was sensitive to Cefuroxime, Cephalexin, Amikacin, and Gentamicin. Streptococcus exhibited sensitivity to the following drugs: Amikacin, Meropenem, Ofloxacin, Vancomycin (92%), and Ciprofloxacin (77%) (34).

The most sensitive drug in general for bacterial cultures in the current study was Amikacin, followed by Meropenem, while other studies indicated that the most sensitive drug was Cefuroxime, followed by Amikacin(34).

Conclusions: The frequency of bacterial growth was 19%. The most frequently isolated bacteria from the cultures were Staphylococcus followed by E. coli. The most sensitive drug was Amikacin followed by Meropenem.

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توزيع البكتريا طبقا لمقاومة المضادات الحيوية في تجرثم البول عند النساء البالغات في مدينة سامراء

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ملخص

الخلفية: تعريف البيلة الجرثومية هو وجود البكتيريا في البول مع عدم وجود مجموعة من علامات وأعراض التهاب المسالك البولية. البكتيريا الأكثر شيوعًا التي قد تسبب البيلة الجرثومية هي الإشريكية القولونية. حوالي 1−5% من النساء الأصحاء في فترة ما قبل انقطاع الطمث و(1.9−9.5%) من النساء الحوامل يعانين من البيلة الجرثومية. أكثر الأدوية الفعالة على البكتيريا نتيجة زراعة البول هي النيتروفورانتوين (98.3%) يليه السيفوروكسيم (89.3%) والكوتريموكسازول (20%).

الهدف والغايات: التعرف على توزيع البكتيريا حسب مقاومة الأدوية بين النساء البالغات المصابات بالبول الجرثومي في مدينة سامراء/ العراق، وتحديد بعض العوامل المؤثرة.

المواد والطرق: كانت الدراسة عبارة عن دراسة وصفية مقطعية أجريت على النساء البالغات (18−44) سنة اللاتي يترددن على مستشفى سامراء العام. تم الحصول على المعلومات الديموغرافية مع نتائج الفحوصات المختبرية وفقًا للاستبيان المعد لذلك. عندما ينمو نوع واحد من البكتيريا في البول مع ما لا يقل عن 100000 وحدة تشكيل مستعمرة لكل ملليلتر، سواء كانت البيلة البولية موجودة أم لا، يتم تحديد مريض يعاني من البيلة الجرثومية بدون أعراض، حتى في حالة عدم وجود أي أعراض لالتهاب المسالك البولية. عندما يكون ذلك مناسبا، تم استخدام التكرارات (عدد الحالات) والنسب المئوية لوصف البيانات إحصائيا. تمت المقارنة بين مجموعات الدراسة باستخدام اختبار مربع كاي. واعتبرت قيم (p) اقل من 0.05 ذات دلالة إحصائية.

النتائج: بلغ معدل نمو البكتيريا في العينة (19%). أعلى تكرار للحالات كان بين الفئة العمرية (28−37) سنة (22.4%). وكانت الحالات الإيجابية أكثر انتشارا بين النساء الحوامل (21.4%) مقارنة بغير الحوامل (13.3%). تمثل المكورات العنقودية أكثر أنواع البكتيريا شيوعا (42%)، يليها الإشريكية القولونية (39%)، الكليبسيلا (11%)، وأخيرا المكورات العقدية (8%). وكان الدواء الأكثر حساسية بشكل عام لنمو البكتيريا هو أميكاسين يليه أدوية الميروبينيم.

الاستنتاجات: كان معدل نمو البكتيريا 19٪. وكانت البكتيريا الأكثر شيوعا المعزولة من المزرعة هي المكورات العنقودية تليها الإشريكية القولونية. وكان الدواء الأكثر حساسية هو الأميكاسين يليه الميروبينيم.

الكلمات الدالة: البيلة الجرثومية، مقاومة الأدوية، سامراء.

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Medicinal Plants Used by Traditional Healers in the Treatment of Gastrointestinal Disorders in Oued Souf Region (southeast of Algeria)

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ABSTRACT

This study aims to analyze indigenous knowledge of medicinal plants used by traditional healers to treat gastrointestinal disorders in the Oued Souf region. Data were collected through open-ended, semi-structured interviews. Various statistical indices, such as UV and ICF, were employed to evaluate quantitative data. The findings reveal that traditional healers utilize 47 medicinal plant species from 22 families for treating gastrointestinal disorders. Lamiaceae and Asteraceae emerge as the most dominant families, with 9 and 7 species, respectively. The most frequently used plant parts were leaves (35%), and the predominant method of preparation was infusion (55%). Among the most popular plants used by local healers were Artemisia herba alba Asso (UV = 0.85) and Juniperus communis (UV = 0.75). The study highlights the significant number and variety of medicinal plants employed by traditional healers to address digestive disorders. Consequently, this research can aid scientists in identifying plants with medicinal properties that may contribute to the development of new medications.

Keywords: Gastrointestinal disorders, Traditional healers; Oued Souf; Medicinal plants; Indigenous knowledge.

INTRODUCTION

The gastrointestinal tract, a highly sensitive human organ, is susceptible to a diverse range of diseases, including parasites, infectious disorders, gastroenteritis, reflux, bloating, constipation, and diarrhea[1,2] . The prevalence of gastrointestinal illness is notably attributed to infections from various bacterial strains, causing up to 3 million preschooler deaths annually[3]. There is a growing interest in traditional medical systems, driven by the need for more efficient treatment. The demand for fundamental scientific research on medicinal plants used in indigenous medical systems has consequently increased. Recognizing the importance of traditional medicine, the World Health Organization (WHO) acknowledges it as the totality of knowledge, skills, and practices based on theories, beliefs, and experiences inherent to various[4,5].

In Algeria, phytotherapy is deeply rooted in local culture, with indigenous knowledge accumulated over decades through practical study. The diverse flora, fostered by Algeria's geographic position and varied climate, has been extensively used to address numerous maladies, especially digestive system problems[6,7]. Despite lifestyle changes and industrialization, local communities in Algeria's Sahara, one of the world's largest deserts, still rely on traditional healers for medical needs[8]. Recognizing the declining transmission of this tradition, it has become crucial to record the historical applications of therapeutic herbs[6].

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Ethnopharmacological studies play a vital role in acquiring and safeguarding ancestral medicinal history. Scientific investigations are necessary to confirm the efficacy claimed by conventional healers and to identify bioactive substances[9,10]. While numerous ethnobotanical studies globally explore traditional remedies for gastrointestinal disorders[11-14], limited details are known about the traditional usage of therapeutic herbs in the Oued Souf region (North Southeast Algeria) for treating digestive system diseases.

This study aims to document and analyze local knowledge of medicinal plants used by traditional healers to treat gastrointestinal disorders in the Oued Souf region. Specific details include the species employed in treatment, the types of gastrointestinal conditions addressed by certain plants, and the parts of the plant used as medication. Ethnomedical indices are utilized to determine the most preferred plants in the study area, with the data serving as a basis for additional phytochemical and pharmacological research.

MATERIAL AND METHODS

Study area

The Oued Souf region is located in the north-southeast part of Algeria, covering a total area of more than 54,573 km² with a population of 504,401 inhabitants. Geographically, it is situated between latitudes 34° 17' 25" north and 7° 42' 41" east. The research location is bordered to the north by the wilayas of Biskra, Khenchela, and Tébessa, to the east by the Tunisian border, to the west by the wilaya of Djelfa, and to the south by the wilaya of Ouargla. The region comprises three distinct zones, including a sandy region that spans the entire Souf area, as well as the eastern and southern parts of Oued-Righ. This area is part of the great eastern erg and has limited agricultural significance (Figure 1)[15].

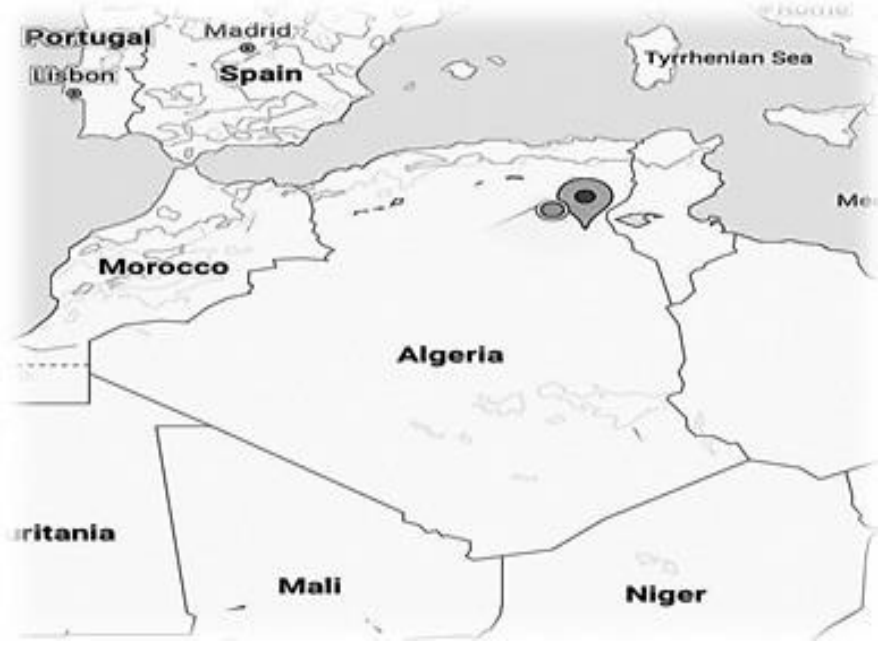

Figure 1. Location of study area (Oued Souf, southeast of Algeria)

Data Collection

This study was conducted from May to September 2022, during which we carried out 20 interviews with traditional healers in the research region, obtaining their permission for participation. To identify medicinal plants used in the treatment of gastrointestinal disorders, we employed semi-structured questionnaires with open-ended questions. The questionnaire, divided into two parts, gathered socio-demographic information (address, age, sex, education level, and years of experience) and details about medicinal plants used for various digestive tract diseases (local name, scientific name, part used, mode of preparation, therapeutic uses, and usage warnings).

Vegetable Resources

During the collection of plant specimens, we sought validation from multiple specialists to ensure result accuracy. The identification of plant samples was confirmed by Professor Youcef Helis from the Scientific and Technical Research Center on Arid Regions C.R.S.T.R.A, Campus of Mohamed Khider University. The scientific and popular names of the medicinal plants were verified using the web database (www.theplantlist.org) and botanical sources on Algerian flora[16,17]. Herbarium specimens of the identified plants were created and stored in the laboratory of Biodiversity and Application of Biotechnology in the Agricultural Field, Faculty of the Sciences of Nature and Life, University of El Oued, Algeria.

Data analysis

The obtained information was statistically examined using metrics such as Use Value (UV) and Informant Consensus Factor (ICF).

Use value (UV)

According to Phillips, Gentry, Reynel, Wilkin, and Gálvez-Durand B[18], UV assessed the relative relevance of a species compared to others and is calculated as: **UV = ΣU/N**, where ***U*** is the number of reports of uses for a certain species, and ***N*** is the total number of informants. A high UV value implies significance, while a low UV value suggests lesser importance than other species[19].

Informant consensus factor (ICF)

ICF measures the degree of knowledge homogeneity among informants. The range is 0 to 1, calculated as: **ICF = (Nur - Nt) / (Nur - 1)**, where ***Nur*** is the number of citations used in each disease category, and ***Nt*** is the number of species used[20].

RESULTS

Demographics data of the responders

In terms of age distribution, the majority of research participants fell within the 41 to 60 age range (50%). Among the total traditional healers, fifteen percent were male, and eighty-five percent were female. The informants exhibited diverse educational backgrounds, ranging from 15% being illiterate to 85% being literate. The results indicate varying levels of expertise among traditional healers, with 45% having the highest proficiency (Table 1).

Table 1. Demographics of survey respondents on medicinal plants used in the treatment of gastrointestinal disorders in Oued Souf region, algeria.

Variable	Categories	Percentage
Sex	Male	85%
	female	15%
Age(years)	<20	0%
	20-40	35%
	41-60	50%
	>60	15%
Educational level	illiterate	15%
	primary level	10%
	middle level	10%
	secondary level	40%
	University level	25%
Experience (years)	10-20	40%
	21-40	45%
	41-60	15%

Utilization of medicinal plants by traditional healers for the treatment of gastrointestinal disorders

Traditional healers in the Oued Souf region utilize 47 species of medicinal plants from 22 botanical families to treat gastrointestinal disorders, as detailed in Table 2. Three plant families are notably significant: Lamiaceae with 9 species, Asteraceae with 7 species, and Apiaceae with 6 species, as illustrated in Figure 2.

Table 2. List of medicinal plants used by traditional healers to treat gastrointestinal disorders in Oued Souf region (north-southeast of Algeria).

N°	Family	Local name	Scientific name	Growth form	Part used	Mode of preparation	Indication	Usage warnings	UV
1	Lamiaceae	Iklil jabel	*Rosmarinus officinalis* L.	Spontaneous	Leaves	Decoction	Gastrointestinal gases	-Is not advised for women who are pregnant or nursing - Causes blood pressure disorders	0.5
		khozama	*Lavandula angustifolia* Mill.	Cultivated	Fruits	Infusion	-Gastrointestinal gases -Gastric disorders	-Leads to male breast development	0.1
		khyata	*Teucrium polium* L.	Spontaneous	All plant	Decoction, Powder	-Gastric ulcer -Diarrhea	-Is not advised for women who are pregnant or nursing -Causes liver disorders	0.2
		Rihan	*Ocimum basilicum* L.	Spontaneous	Leaves	Decoction	-Gastrointestinal gases -Gastric disorders	-Increased bleeding -Causes liver disorders	0.55
		Zaater	*Thymus vulgaris* L.	Spontaneous	All plant	Infusion	-Gastrointestinal gases - Diarrhea	-Is not advised for women who are pregnant or nursing	0.25
		Mardakoch	*Origan marjolaine* L.	Spontaneous	Leaves	Decoction	-Gastric ulcer -Abdominal pain	-Is not advised for: children, women who are pregnant or nursing	0.05
		Maryot	*Marrubium vulgare* L.	Spontaneous	leaves	Infusion	-Indigestion -Gastrointestinal gases	-Is not advised for: women who are pregnant or nursing, children	0.1
		Miramia	*Salvia officinalis* L.	Spontaneous	Stems, Leaves	Infusion	- Diarrhea -Abdominal pain	-Is not advised for: diabetics, women who are pregnant or nursing -Causes liver disorders	0.1

N°	Family	Local name	Scientific name	Growth form	Part used	Mode of preparation	Indication	Usage warnings	UV
		Naanaa	*Mentha crispata* L.	Cultivated	Leaves	Infusion	-Gastrointestinal gases	-Is not advised for diabetics, women who are pregnant or nursing, children	0.25
2	Asteraceae	Babonj	*Matricaria chamomilla* L.	Cultivated	Flowers	Infusion	-Gastric disorders| -Abdominal pain	- It is not permitted for surgical patients	0.3
		Chih	*Artemisia herba alba Asso*	Spontaneous	Leaves	Infusion	-Indigestion -Gastrointestinal gases -Irritable bowel syndrome	-Induces sleeplessness -leads to vomiting	0.85
		Keset hindi	*Saussurea costus* L.	Spontaneous	Roots	Infusion, powder	-Gastric ulcer	-Causes blood pressure disorders -Is not advised for women who are pregnant or nursing	0.05
		Kartofa	*Anacyclus valentinu* L.	Spontaneous	Seeds	Infusion	- Diarrhea -Irritable bowel syndrome -Gastric ulcer	-Is not advised for women who are pregnant or nursing	0.2
		Hindba	*Cichorium intybus* L.	Spontaneous	Roots, Leaves, Flowers	Infusion	-Constipation -Abdominal pain -Gastrointestinal gases	-leads to gallbladder problems -It is not permitted for surgical patients	0.05
		Meraret henech	*Entyraea centarium* L.	Spontaneous	All plant	Decoction, Powder	-Gastrointestinal gases -Hemorrhoids	-Is not advised for women who are pregnant or nursing, children -Causes gastric ulcers	0.1
		Dgeft	*Artemisia campestris* Scop.ex Steud	Cultivated	Leaves	Infusion	- Diarrhea -Abdominal pain	-Is not advised for women who are pregnant or nursing -Causes gastric ulcers	0.25
3	Apiaceae	Heltit	*Ferula assa –foetida* L.	Spontaneous	All plant	Decoction	-Gastric ulcer -Gastrointestinal gases -Irritable bowel syndrome	-Causes blood pressure disorders	0.05

N°	Family	Local name	Scientific name	Growth form	Part used	Mode of preparation	Indication	Usage warnings	UV
		Deriga	*Ammodaucus leucotrichus* Coss. & Durieu	Spontaneous	Seeds	Infusion, decoction	-Irritable bowel syndrome -Abdominal pain gastrointestinal gases -Constipation	- The dosage must be followed	0.15
		Krefs	*Apium graveolens* L.	Cultivated	Seeds	Infusion	-Gastric ulcer -Abdominal pain -Constipation	-Causes blood pressure disorders	0.1
		Kesber	*Coriandrum sativum* L.	Cultivated	Leaves, Seeds	Infusion	-Gastrointestinal gases	-Is not advised for Diabetics, women who are pregnant or nursing -Causes blood pressure disorders	0.1
		Kemun	*Cuminum cyminum* L.	Cultivated	Seeds	Decoction	-Gastrointestinal gases -Irritable bowel syndrome - Diarrhea	-Causes gastric ulcers -Is not advised for women who are pregnant or nursing	0.65
		Yenson	*Pimpinella anisum* L.	Spontaneous	Seeds	Infusion	-Gastric ulcer -Constipation	-Is not advised for Diabetics	0.45
4	Fabaceae	Helba	*Trigonella foenum-graecum* L.	Cultivated	Seeds	Decoction	-Gastric disorders -Constipation	-Is not advised for women who are pregnant or nursing,children	0.45
		Erek sos	*Glycyrrhiza glabra* L.	Spontaneous	Roots	Decoction, Powder	-Chronic inflammatory disorders -Gastric ulcer	-Causes blood pressure disorders -Is not advised for women who are pregnant or nursing	0.15
		Sena meki	*Senna alexandrina* Mill.	Spontaneous	Leaves	Infusion, decoction	-Constipation	-Causes severe diarrhea	0.5
5	Cupressaceae	Debegh	*Thuja occidentalis* L.	Spontaneous	All plant	Infusion	-Chronic inflammatory disorders	-Causes severe diarrhea	0.35
		Araar	*Juniperus communis* L.	Spontaneous	Leaves	Infusion	- Diarrhea -Gastrointestinal gases	-Leads to renal disorders -Causes blood pressure disorders -Is not advised for Diabetics	0.75

N°	Family	Local name	Scientific name	Growth form	Part used	Mode of preparation	Indication	Usage warnings	UV
6	Lauraceae	Rend	*Laurus nobilis* L.	Spontaneous	Leaves	Infusion	-Abdominal pain - Diarrhea -Gastrointestinal gases	-Causes blood pressure disorders -Is not advised for women who are pregnant or nursing	0.15
		Kerfa	*Cinnamomum verum* J.Presl	Cultivated	Stems	Decoctio	-Gastrointestinal gases - Diarrhea	-Increased bleeding -Causes liver disorders -Is not advised for women who are pregnant or nursing	0.05
7	Zingiberaceae	Zenjabil	*Zingiber officinale* Roscoe	Cultivated	Roots	Decoction	-Irritable bowel syndrome -gastric ulcer	-Not take it on an empty stomach	0.25
		Kerkum	*Curcuma longa* L.	Cultivated	Stems	Infision	-Chronic inflammatory disorders	-Increased bleeding -Causes blood pressure disorders -Is not advised for Diabetics	0.1
8	Chenopodiaceae	Ktef	*Atriplex halimus* L.	Spontaneous	Leaves, Seeds	decoction, Powder	-Constipation	-Causes gastric ulcers	0.05
		Demran	*Traganum nudatum* Delile	Spontaneous	All plant	Infusion, decoction	- Diarrhea -Hemorrhoids -Gastrointestinal gases	- The dosage must be followed	0.15
9	Phyllanthaceae	Amlej	*Phyllanthus emblica* L.	Spontaneous	Seeds	Infusion	-Constipation -Gastric ulcer	-Is not advised for women who are pregnant or nursing,Diabetics	0.05
10	Crassulaceae	Serra	*Centella asiatica*.L.	Spontaneous	Roots	Infusion	-Chronic inflammatory disorders	- The dosage must be followed	0.1
11	Amaryllidaceae	Besbess	*Foeniculum vulgare* Mill.	Spontaneous	Seeds	Infusion	-Constipation -Gastrointestinal gases	-Is not advised for women who are pregnant or nursing -Increased bleeding	0.7
12	Lythraceae	Henna	*Lowsonia inermis* L.	Cultivated	leaves , Flowers	Decoction	- Diarrhea	-Is not advised for women who are pregnant or nursing	0.2
13	Theaceae	Chee	*Camellia sinensis* L.	Cultivated	Leaves	Decoction	-Chronic inflammatory disorders	-Causes gastric ulcers -Induces sleeplessness	0.1

N°	Family	Local name	Scientific name	Growth form	Part used	Mode of preparation	Indication	Usage warnings	UV
14	Pinaceae	Senober	*Pinus gerardiana* Wall. Ex D.Don	Spontaneous	Stems	Infusion	-Abdominal pain -Chronic inflammatory disorders -Gastrointestinal gases	- Weight gain	0.1
15	Verbenaceae	Louiza tizana	*Aloysia citrodora* Palau	Cultivated	Leaves	Infusion	-Gastrointestinal gases	-Causes thyroid malfunction	0.15
16	Anacardiaceae	Mestka hora	*Pistacia lentiscus* L.	Cultivated	All plant	Powder	-Gastric ulcer -Chronic inflammatory disorders	-Is not advised for women who are pregnant or nursing, children	0.1
17	Caesalpinioi-deae	kherob	*Ceratonia siliqua* L.	Cultivated	Fruits	Infusion	- Diarrhea -Irritable bowel syndrome	-Is not advised for women who are pregnant or nursing	0.1
18	Apocynaceae	Karenka	*Calotropis procera* A.T.Aiton	Cultivated	leaves, Flowers, Roots	Powder	-Gastric ulcer - Diarrhea	- The dosage must be followed -Is not advised for women who are pregnant or nursing	0.05
19	Rutaceae	Fijel	*Ruta graveolens* L.	Spontaneous	Leaves	Infusion	-Gastrointestinal gases -Abdominal pain -Chronic inflammatory disorders	-Is not advised for women who are pregnant or nursing, children	0.2
20	Rhamnaceae	Sedra	*Ziziphus spina-christi* L.	Spontaneous	Leaves	Infusion	- Gastrointestinal gases - Constipation - Gastric ulcer	-Is not advised for women who are pregnant or nursing, children	0.2
21	Nitrariaceae	Hermel	*Peganum harmala* L.	Spontaneous	Seeds	Infusion	-Abdominal pain	-Is not advised for women who are pregnant or nursing -Causes gastric ulcers	0.15
22	Tamaricaceae	Terfa	*Tamarix aphylla* L.	Spontaneous	All plant	Infusion, decoction	-Constipation	-Is not advised for women who are pregnant or nursing, children	0.1

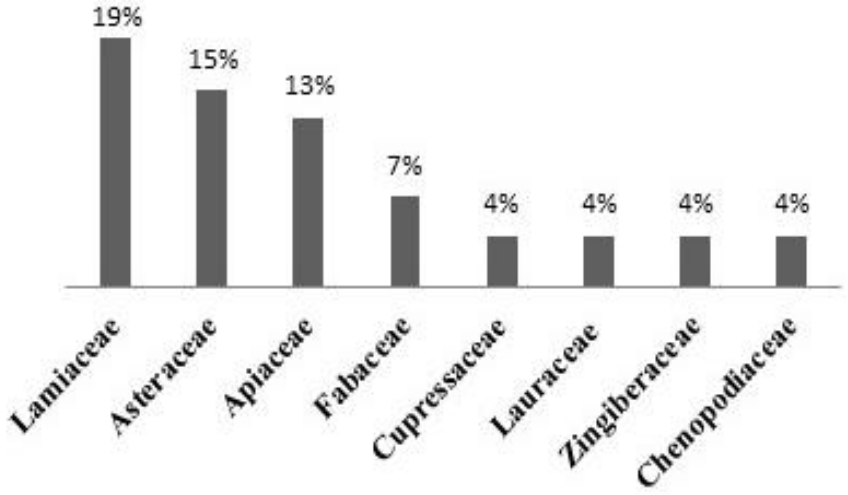

Figure 2. The majority of listed botanical families.

Most frequently utilized species

The most frequently utilized species by traditional healers in the treatment of digestive system disorders include Artemisia herba alba Asso (17 citations), Juniperus communis (15 citations), Foeniculum vulgare (14 citations), Cuminum cyminum (13 citations), Ocimum basilicum (11 citations), Senna alexandrina, and Rosmarinus officinalis (10 citations), as depicted in Figure 3.

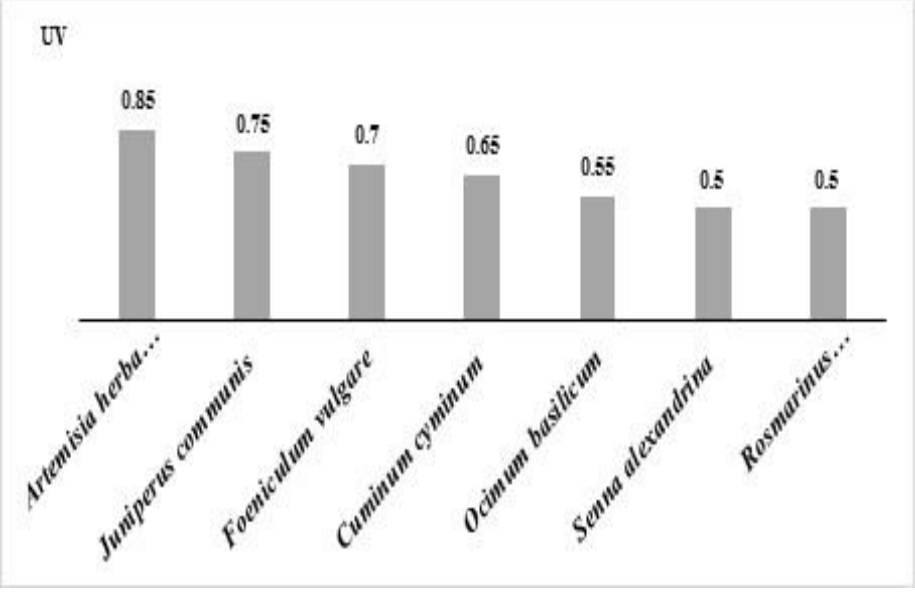

Figure 3. Most frequently utilized species.

Used part

Results indicate that leaves are the most frequently utilized part (35%), followed by seeds (20%), the entire plant (15%), roots (11%), stems (8%), flowers (7%), and fruits (4%) (Figure 4).

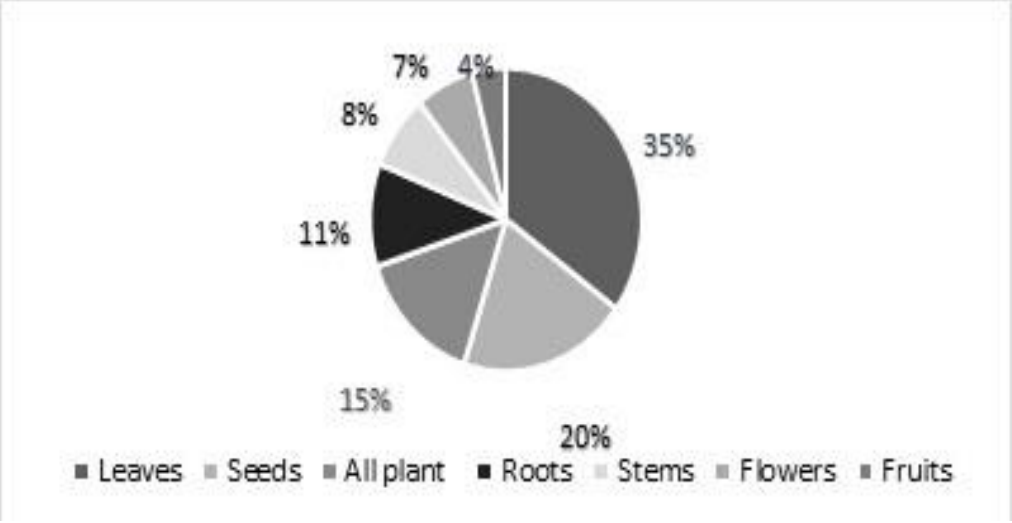

Figure 4. Frequency of plant parts used for treating gastrointestinal disorders.

Method of preparation

The study revealed that the infusion method was the most frequently indicated for preparing herbal remedies (55%), followed by decoction (32%) and powder formulations (13%) (Figure 5).

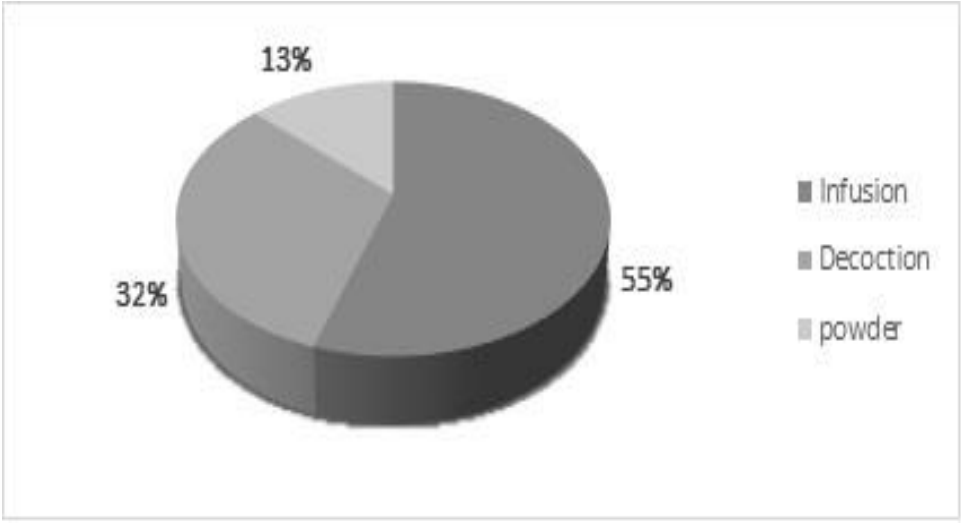

Figure 5. Frequency of several preparation methods for the treatment of gastrointestinal disorders.

Therapeutic uses

Based on the information provided by the respondents, ailments were classified into 10 disorders, with gastrointestinal gases (23%) being the condition most frequently treated with the indicated medicinal plants. This was followed by gastric ulcer and diarrhea (14% each), constipation and abdominal pain (12% each), chronic inflammatory disorders (9%), irritable bowel syndrome (8%), gastric disorders (4%), indigestion, and hemorrhoids (2% each) (Figure 6).

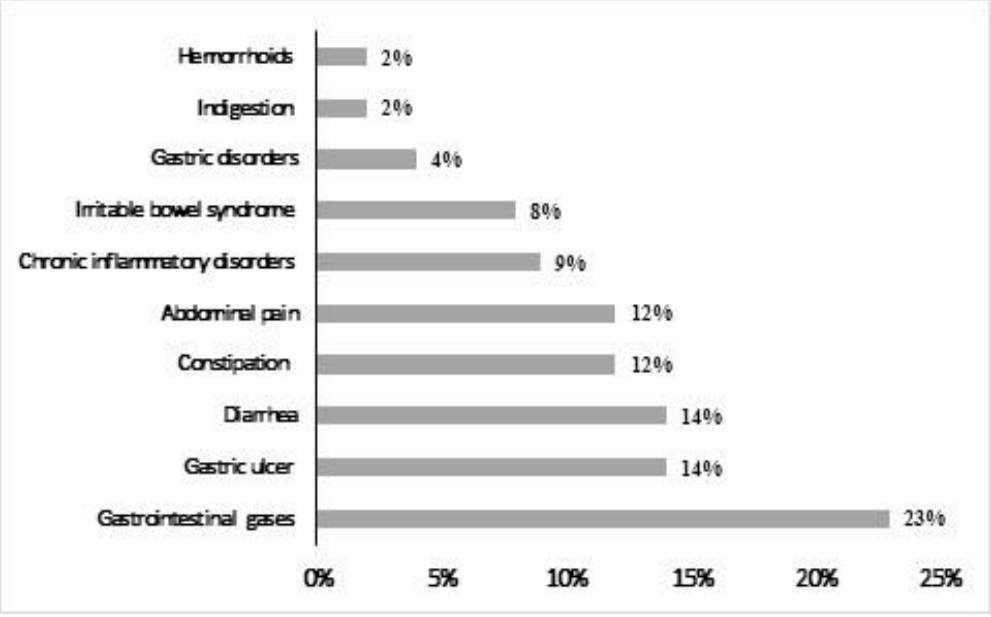

Figure 6. Gastrointestinal disorders treated by medicinal plants in the research area.

Usage warnings

The most crucial warnings provided by traditional healers emphasize that using medicinal herbs to treat digestive ailments is not advised for pregnant or nursing women (33%), children (11%), and individuals with diabetes and blood pressure disorders (9% each). Other cautions include the potential to cause gastric ulcers (7%), liver disorders, increased bleeding, and the importance of following specified dosages (5% each). Additional precautions include avoiding use by surgical patients, as it may cause severe diarrhea and induce sleeplessness (3% each), and it should not be taken on an empty stomach to prevent vomiting, gallbladder problems, male breast development, weight gain, thyroid malfunction, and renal disorders (1% each) (Figure 7).

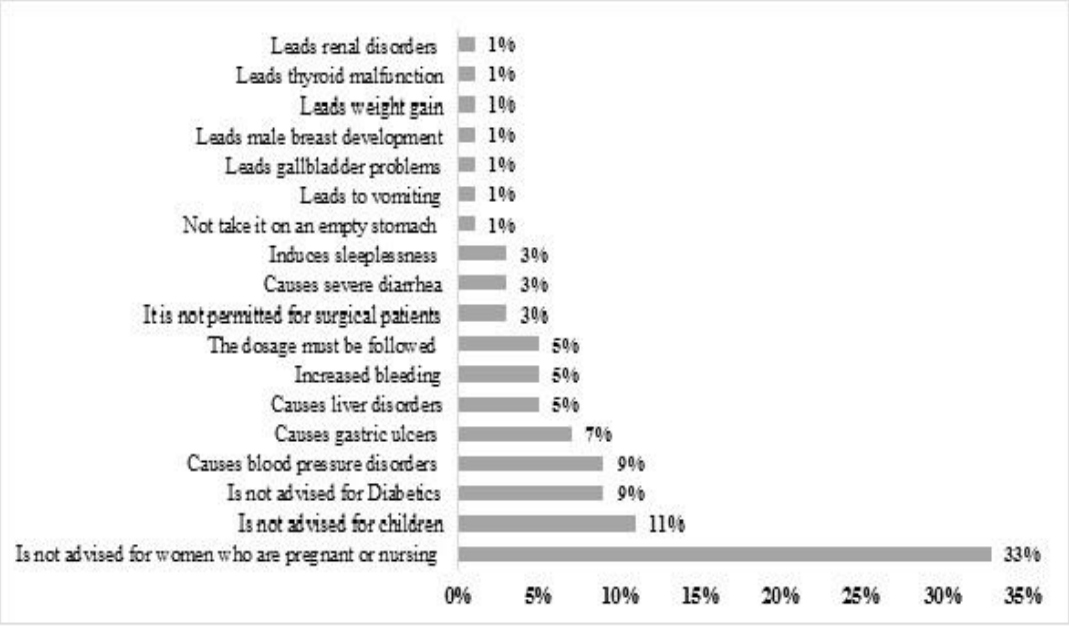

Figure 7. Usage warnings given by traditional healers

Statistical data analysis

Use value UV

The medicinal plants with the highest and lowest usage reports also exhibit the highest and lowest respective use values. In this study, Artemisia herba alba Asso demonstrated the greatest UV value (0.85), while several plants, including Atriplex halimus and Calotropis procera, had the lowest UV value (0.05) (Table 2).

Informant consensus factor (ICF)

The Informant Consensus Factor (ICF) was determined by categorizing all disorders into ten categories in the current study. Hemorrhoids and indigestion showed the highest ICF values (1 each), while gastrointestinal gases had a value of 0.55 (refer to Table 3).

Table 3. Value of informant consensus factor (ICF) for each disease category.

Disease categories	Nur	Nt	ICF
Gastrointestinal gases	21	10	0.55
Gastric disorders	4	3	0.33
Gastric ulcer	13	9	0.33
Diarrhea	13	8	0.41
Abdominal pain	11	7	0.4
Indigestion	2	1	1
Constipation	11	7	0.4
Irritable bowel syndrome	7	5	0.33
Chronic inflammatory disorders	8	5	0.42
Hemorrhoids	2	1	1

DISCUSSION

Though traditional medicine is often associated with a particular gender, it is practiced by both men and women in some cultures. In the current study, a higher number of male respondents were interviewed compared to female respondents. A comparable study by Khoja, Andrabi, and Mir[20] indicated that 34 male healers (70.83%) and 14 female healers (29.17%) were involved. Additionally, research in the M'sila region of Algeria revealed a predominantly male participation in traditional medicine[21]. The sociocultural framework of the society, actual circumstances, informants' commitment, and associated sociocultural boundaries are factors influencing ethnobotanical surveys.

The majority of participants in the present study were aged 41-60 (50%), with the majority of healers (85%) being literate. It appears that the number of reported species is correlated with the informants' ages. Younger individuals, exposed to modern education, may have decreased interest in learning about and applying ethnomedical practices. Simultaneously, as science and technology advance rapidly, younger generations are adopting new customs[22]. Additionally, 45% of healers have between 21 and 40 years of experience. Bouasla and Bouasla6 emphasize that experience gained with age provides older individuals with more knowledge. Furthermore, Kadir, Sayeed, and Mia[23] found that the majority of healers (33.36%) have 10–20 years of relevant expertise from their ethnopharmacological assessment.

This study reports the utilization of 47 medicinal plant species from 22 families by traditional healers for treating gastrointestinal disorders in the Oued Souf region. Lamiaceae, with 9 species, emerges as the most utilized plant, followed by Asteraceae (7 species), Apiaceae (6 species), and Fabaceae (3 species). These findings contrast with those of Kadir, Sayeed, and Mia[23], who claimed that the Fabaceae family accounted for the majority of medicinal plants used by traditional healers in Bangladesh. This discrepancy highlights the considerable taxonomic diversity of medicinal plants in our study area, underscoring the wealth of knowledge regarding their application in traditional gastrointestinal treatment[24]. Furthermore, Lamiaceae is notable for its high content of phenolics and flavonoids, contributing to its elevated antioxidant levels, as demonstrated in previous research[25].

The most cited species in our study are *Artemisia herba alba Asso* and *Juniperus communis* (17-15 citations). The widespread usage of these species by respondents for various ailments can be attributed to their familiarity and frequent employment. However, it is crucial to note that intensive usage and overuse of these species may jeopardize their survival, impacting the region's biodiversity[6].

Results indicate that leaves are the most frequently utilized plant part (35%). This aligns with several investigations emphasizing the significance of leaves in developing remedies, as highlighted by Abdulsalami, Mudi, Daudu, Aliyu, Adabara, and Hamzah[22]. The effectiveness of leaves in treating illnesses may be attributed to the various bioactive components they contain, as leaves actively participate in photosynthesis, making them a crucial component of several herbal remedies[26].

The study revealed that the infusion method was most commonly indicated (55%). Traditional healers believe that infusion is the most effective method for preparing medicinal extracts to treat gastrointestinal diseases because it maintains the therapeutic characteristics of the extract, allowing for the secure extraction of active ingredients[27]. The simplicity of preparation and administration makes decoction and infusion in water popular techniques, as highlighted in previous research[28,29].

Gastrointestinal gases (23%) represent the most frequently treated condition with the indicated medicinal plants. Several plants have demonstrated robust biological defenses against a range of digestive illnesses. Consequently, delving into the biological research of some medicinal plants used by Oued Souf healers to treat

gastrointestinal disorders becomes particularly intriguing.

Traditional healers emphasize a crucial piece of advice by prohibiting the use of medicinal herbs for pregnant women (33%). It is erroneous and deceptive to assume that herbal treatments are exceptionally safe and devoid of side effects, as is commonly believed. Herbs have been shown to induce various unpleasant or unfavorable responses, some of which have the potential to be lethal or cause severe injuries and other life-threatening conditions. It is crucial to remember that larger dosages of therapeutic plants can occasionally have detrimental consequences[30].

In the current study, Artemisia herba-alba Asso and Juniperus communis have the highest UV value (0.85) due to their diverse therapeutic characteristics. Conversely, low usage values (UV) for medicinal plants suggest that access to or knowledge of those particular plants may be at risk[31,32]. Hemorrhoids and indigestion each have the highest ICF values (1). A high ICF score (1.0 or near 1) indicates the usage of a relatively small number of plant species by a significant majority of informants[33].

Most frequently, plants such as Foeniculum vulgare, Cuminum cyminum, Coriandrum sativum, Ammodaucus leucotrichus, Rosmarinus officinalis, Ocimum basilicum, Mentha crispata, Entyraea centarium, Aloysia citrodora, Ruta graveolens, and Traganum nudatum were claimed to be helpful for treating gastrointestinal gas. The informants' consensus on using a specific plant species to treat various ailments is reflected in the high ICF value. This suggests that these plants may contain physiologically active components[34]. Foeniculum vulgare (Apiaceae) is a well-known plant with significant therapeutic value, particularly for treating gastrointestinal disorders[35]. Cumin seeds are believed to have carminative properties, and according to tradition, the plant may be effective in treating a variety of conditions, including diarrhea, jaundice, dyspepsia, indigestion, and stomach discomfort[36,37].

CONCLUSION

The aim of the present study was to identify potential medicinal plants in the Oued Souf region (North Southeast Algeria) that traditional healers may use to treat various gastrointestinal disorders.

Our survey yielded a wealth of data, clearly demonstrating that traditional healers in the Oued Souf region employ numerous medicinal plants for treating various digestive system ailments. A total of 47 medicinal plant species from 22 families were documented in this research, with Lamiaceae and Asteraceae being the most common families. Plant leaves were predominantly used to address GI-related problems, and infusion emerged as the most widely employed conventional preparation technique in the area. It is crucial to document the preservation of traditional knowledge before it diminishes from the region, where it is disappearing at an alarming rate. While preliminary research on these medicinal plants has shown their effectiveness, further investigation is necessary, particularly to ensure the safe use of these plants in therapeutic procedures.

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النباتات الطبية المستخدمة من طرف المعالجين التقليديين في علاج اضطرابات الجهاز الهضمي في منطقة واد سوف (جنوب شرق الجزائر)

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ملخص

هدفت هذه الدراسة إلى توثيق وتحليل المعرفة الأصلية للنباتات الطبية التي يستخدمها المعالجون التقليديون لعلاج اضطرابات الجهاز الهضمي في منطقة وادي سوف (شمال الشرق الجزائري)، تم إجراء الاستطلاع من مايو إلى سبتمبر 2022، وفيه استجوب 20 معالجًا تقليديًا باستخدام أسئلة مفتوحة واستبيان شبه منظم. يتضمن النموذج المعلومات الاجتماعية والديموغرافية للمعالج والأسماء المحلية والعلمية للنبات الطبي، الأجزاء المستخدمة منه وطرق تحضيره، تحذيرات الاستخدام. لتقييم البيانات التي تم جمعها، تمت دراسة عاملين هما Usage Value و Informant Consensus Factor. أظهرت النتائج التي توصلنا إليها أن المعالجين التقليديين في منطقة واد سوف يستخدمون 47 نوعًا من النباتات الطبية من 22 عائلة لعلاج اضطرابات الجهاز الهضمي. كما كشفت نتائج التحقيق أن العمر والجنس والمستوى التعليمي وسنوات الخبرة جميعها لها تأثير على مدى تكرار استخدام النباتات الطبية. تعتبر كل من Lamiaceae، Asteraceae، أكثر العائلات استعمالا بمعدل 9 و 7 أنواع على التوالي. كانت الأجزاء النباتية الأكثر استخدامًا هي الأوراق (35٪)، والغليان هي الطريقة الأفضل في تحضير المستخلص العشبي (55٪). بالإضافة إلى ذلك، كان النبات الأكثر شيوعًا الذي استخدمه المعالجون المحليون هو Asso *Artemisia herba alba* (UV = 0.85). كما تم الاتفاق على أن عسر الهضم والبواسير هي أكثر اضطرابات الجهاز الهضمي التي يتم علاجها (FIC =1 لكل منهما). ومنه فان المعالجين التقليديين في منطقة وادي سوف على دراية تامة بالنباتات الطبية واستخداماتها. ويمكن للباحثين والعلماء العثور على نباتات ذات صفات طبية قد تكون مفيدة في ابتكار أدوية جديدة باستخدام هذا المسح العرقي النباتي.

الكلمات الدالة: اضطرابات الجهاز الهضمي، المعالجون التقليديون، وادي سوف، النباتات الطبية، المعرفة التقليدية، الجزائر.

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The Relationship between Levels of Zinc and Copper and Insulin Resistance in Polycystic Ovary Syndrome Patients in Homs

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ABSTRACT

Objective: To investigate the association between zinc and copper levels and insulin resistance, a key pathological mechanism of Polycystic Ovary Syndrome (PCOS), and to compare these levels with those of healthy subjects in Homs.

Methods: The study included 63 female patients newly diagnosed with PCOS, prior to treatment at Al-Basil Hospital in Homs, Syria, along with 25 healthy subjects of similar age. Blood samples were collected using dry tubes for laboratory measurements of zinc, copper, glucose, and insulin hormone levels. Subsequently, the HOMA-IR and QUICKI indices were calculated.

Results: In the patients' group, serum zinc levels were significantly lower (p-value=0.000), and serum copper levels were significantly higher (p-value=0.000) compared to healthy subjects. Among patients with insulin resistance, serum zinc levels were significantly lower (p-value=0.004), and serum copper levels were significantly higher (p-value=0.000) compared to patients without insulin resistance. Patients without insulin resistance had significantly lower serum zinc levels (p-value=0.000) and significantly higher serum copper levels (p-value=0.000) compared to healthy subjects. There was a positive correlation between copper and HOMA-IR (r=0.572**, p-value=0.000), and a negative correlation between zinc and HOMA-IR (r=-0.865**, p-value=0.000).

Conclusion: The imbalance in zinc and copper levels appears to play a role in the development of PCOS, both in relation to insulin resistance and potentially as an independent factor.

Keywords: Polycystic ovary syndrome, Zinc, Copper; Insulin resistance.

1. INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a hormonal disorder that commonly affects women of reproductive age, leading to hirsutism and fertility issues primarily due to anovulation [1]. The diagnosis of PCOS is based on the Rotterdam Criteria, which necessitates the presence of two of three distinctive features: oligo or anovulation, ovarian cysts detected by ultrasound, and clinical and/or biochemical signs of hyperandrogenism [2,3]. In PCOS, there is an increase in Leuteinizing Hormone (LH) and a decrease in Follicle Stimulating Hormone (FSH), resulting in elevated levels of androstendione that remains unconverted to estrogen. The excessive androgen leads to the formation of small follicles that are incapable of maturation [4]. This syndrome presents several complications, with the most significant ones including cardiovascular issues, infertility, and mood disorders [5]. Insulin Resistance (IR) occurs when the sensitivity and response of liver, adipose, and skeletal muscle cells to normal levels of insulin are reduced. IR stands out as one of the most prominent pathological mechanisms observed in PCOS [6]. The elevated levels of insulin associated with IR increase the action of LH,

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increase the action of LH, which in turn stimulates Theca cells, ultimately resulting in increased production of androgens [7]. Zinc plays an essential role in the body, particularly in insulin secretion and sensitivity [8]. Inside pancreatic β cells, insulin pairs with two zinc ions in secretory granules to form the $ZincO_2$-Insulin6 complex, which is crucial for hormone secretion [9]. The expression of Zinc transporters (ZnTs and ZIPs) in pancreatic β cells directly influences insulin secretion [10]. Additionally, Zinc can modulate the activity of proteins in the insulin signaling pathway, ultimately enhancing glucose uptake [11]. Copper is a vital mineral essential for the proper functioning of most organisms, as it facilitates the movement of electrons within biological molecules [12]. However, an excess of copper can lead to the generation of free radicals, particularly reactive oxygen species (ROS), which in turn can cause impairments in insulin signaling pathways [13].

2. RESULTS AND DISCUSSION

Table 1 presents the demographic characteristics of the study participants. The average age in the patient group was (28.68 ± 7.22) years, and in the control group, it was (28.84 ± 7.83) years for comparison purposes. Based on HOMA-IR values, the patient group was divided into two subgroups using a cutoff value of HOMA-IR ≥ 2.5 to indicate the presence of insulin resistance (IR). Our findings revealed that (65.08%) of patients exhibited insulin resistance, while (34.92%) did not (refer to Fig. 1). The average age in the insulin-resistant patient subgroup was (29.56 ± 7.54) years, whereas in the subgroup without insulin resistance, it was (27.05 ± 6.43) years.

Table 1. Demographic data of subjects included in the study.

Demographic Characteristics	Values (mean ± SD)			
	Patients Group (63)			Control Group (25)
	Patients with IR subgroup (41)	Patients without IR subgroup (22)	Total (63)	
Age (years)	29.56 ± 7.54	27.05 ± 6.43	28.68 ± 7.22	28.84 ± 7.83
BMI (Kg/m^2)	26.13 ± 4.32	24.99 ± 4.39	25.73 ± 4.35	25.38 ± 4.90
WHR	0.80 ± 0.07	0.77 ± 0.07	0.79 ± 0.07	0.74 ± 0.06

*BMI: Body Mass Index = weight (kg)/height (m^2), IR: Insulin Resistance, WHR: Waist-Hip Ratio = waist (cm)/hip (cm)

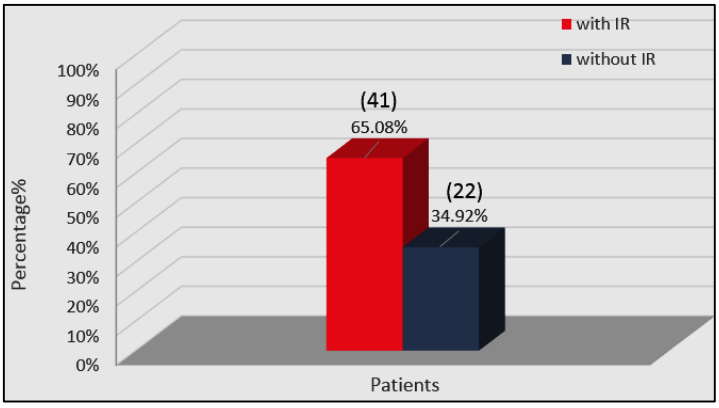

Figure 1. Distribution of patients according to the presence of IR

2.1. Distribution of study samples according to the status of zinc and copper in the serum

Table 2 displays the distribution of the study samples based on the serum levels of zinc and copper, as illustrated in Fig. 2 and Fig. 3.

Table 2. Distribution of study samples according to the status of zinc and copper in the serum.

	Zinc Deficiency	Normal Zinc	Excess Zinc	Copper Deficiency	Normal Copper	Excess Copper
Control Group (n=25)	0% (n=0)	100% (n=25)	0% (n=0)	0% (n=0)	100% (n=25)	0% (n=0)
Patients Group (n=63)	95.24% (n=60)	4.76% (n=3)	0% (n=0)	0% (n=0)	3.17% (n=2)	96.83% (n=61)

*n: number

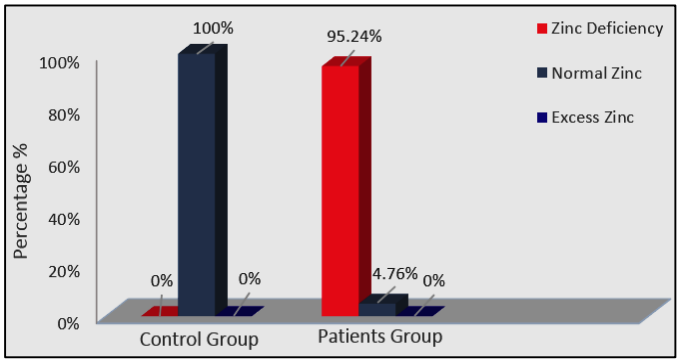

Figure 2 Distribution of study samples according to the status of zinc in the serum

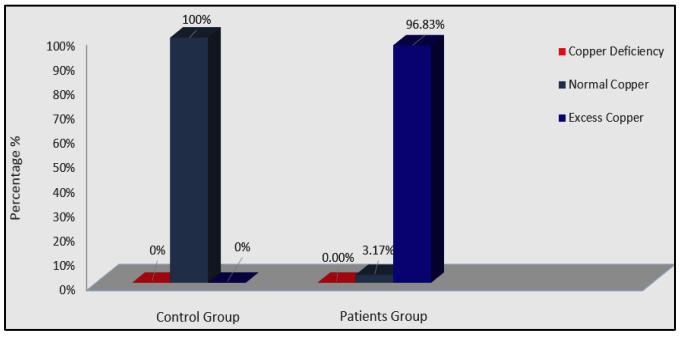

Figure 3. Distribution of study samples according to the status of copper in the serum

2.2. The comparison of the study parameter values between Control Group and Patients' Group

Table 3 presents a comparison of parameter values between the Control Group and Patients' Group. The Control Group demonstrated significant differences in serum zinc values and QUICK index, favoring this group. Conversely, significant differences were observed in serum copper values and HOMA-IR, favoring the Patients' Group.

Table 3. Comparison of the study laboratory parameters between the patient group and the healthy group.

Laboratory Parameters	Values (mean ± SD)		*p*-value
	Patients Group (n = 63)	Control Group (n = 25)	
Zinc (µg/dL)	54.14 ± 10.07	89.06 ± 11.59	0.000
Copper (µg/dL)	189.60 ± 33.26	115.13 ± 20.24	0.000
HOMA-IR [a]	2.33 ± 0.85	1.26 ± 0.70	0.000
QUICK [b]	0.34 ± 0.02	0.38 ± 0.05	0.000

a: homeostatic model assessment for insulin resistance. b: quantitative insulin sensitivity check index.

Based on the findings from Table 2 and Table 3, it was observed that patients exhibited a deficiency in zinc and an excess of copper, while these imbalances were not observed in the healthy subjects. Consequently, it can be inferred that factors related to the environment and dietary patterns significantly influence the levels of zinc and copper. It is worth noting that factors common to both healthy individuals and patients do not predominantly contribute to these deficiencies. The potential factors influencing diet- and environment-related zinc deficiencies may include: 1) The supplementation of calcium can hinder the absorption of zinc. 2) Vegetarian patients with a diet high in phytates may experience zinc deficiency, as phytates are known to bind zinc and inhibit its absorption. 3) Non-heme iron supplements may reduce zinc absorption, unlike heme iron. 4) Deficiency in the ZnT1 transporter, which is responsible for transporting zinc from intestinal cells into the bloodstream. 5) Deficiency of the Zip4 transporter, which is essential for the absorption and transportation of zinc from the intestine through epithelial cells [14]. Factors influencing copper excess deficiency related to diet and environment include: 1) Use of copper pipes for delivering drinking water to households in certain regions. 2) Preparation of food using copper pots. 3) Reduced capacity of the liver to eliminate excess copper [15]. The primary cause of zinc deficiency and copper excess is associated with polycystic ovary syndrome (PCOS) and its pathogenesis, attributed to several factors related to oxidative stress and the inflammatory response commonly observed in PCOS patients: 1) Circulating zinc binds to albumin and copper binds to ceruloplasmin in the bloodstream. The imbalance between oxidative and reductive factors in PCOS patients leads to the dissociation of these metals from their binding forms, resulting in an abundance of free copper that facilitates the transport and displacement of zinc ions into tissues, thereby reducing zinc levels [16]. 2) The inflammatory state [17] and the presence of inflammatory cytokines (IL-6, TNF-α, IL-1, IFN-gamma) regulate the production of ceruloplasmin and inhibit albumin production, consequently leading to decreased zinc levels and increased copper levels [18]. 3) In the presence of pro-inflammatory cytokines, Forkhead box protein O1 (FOXO1) enhances the antioxidant response, leading to upregulation of Zip14 in the liver, causing a decrease in zinc. Moreover, it participates in the synthesis of ceruloplasmin [19]. Our result supports the findings of several previous studies [20-22], and disagrees with a study that found no variation in the levels of these two elements;

this may be due to the small number of samples in their study (47 patients) [23].

2.3. The comparison of the study parameter values between Patients with Insulin Resistance Group (Group 1) and Patients without Insulin Resistance Group (Group 2)

Table 4 presents a comparison of study parameter values between two groups: Patients with Insulin Resistance (Group 1) and Patients without Insulin Resistance (Group 2). Significant differences were observed, with Group 2 showing higher serum zinc values and QUICK index, while Group 1 exhibited significant differences favoring copper and HOMA-IR. The greater zinc deficiency in Group 1 is attributed to its disruption of the insulin signaling pathway, a key factor in the development of insulin resistance. Zinc plays multiple roles, such as stimulating the phosphorylation of the β subunit of the insulin receptor [24], activating PI3K and AKT to promote glucose transport into cells, inhibiting PTEN (which facilitates PI3P dephosphorylation), and regulating the metabolism of glycogen and glucose conversion through the phosphorylation of GSK and inhibition of its action [25], as well as stimulating the phosphorylation of FOXO1 to regulate glucose production [26]. Similarly, excess copper in Group 1 leads to disruptions in the insulin signaling pathway. Copper's conversion from Cu+2 to Cu+1 generates reactive oxygen species (ROS), which inhibit insulin-stimulated glucose uptake and cause defective insulin signaling by phosphorylating the serine/threonine sites of the insulin receptor [27], resulting in decreased transcription of the GLUT-4 gene and reduced GLUT-4 expression, ultimately contributing to insulin resistance [28]. Our findings align with several previous studies [29,30].

Table 4. Comparison of the laboratory parameters between the Patients with IR Group and the Patients without IR Group

Laboratory Parameters	Values (mean ± SD)		*p*-value
	Patients with IR (Group1) (n=41)	Patients without IR (Group2) (n=22)	
Zinc (µg/dl)	51.52 ± 10.49	59.01 ± 7.16	0.004
Copper (µg/dl)	204.62 ± 31.92	161.59 ± 7.90	0.000
HOMA-IR	2.88 ± 0.33	1.29 ± 0.48	0.000
QUICK	0.32 ± 0.004	0.37 ± 0.02	0.000

*n= number

a: homeostatic model assessment for insulin resistance. b: quantitative insulin sensitivity check index.

(Table 5) shows Pearson test.

Table 5. Correlation coefficient in patienrs with insulin resistance group

	HOMA-IR		QUICK		Zinc		BMI		WHR	
	r	***p***	**r**	***p***	**r**	***p***	**r**	***p***	**r**	***p***
Zinc	- 0.865**	0.000	0.851*	0.000	1	-	- 0.146	0.361	0.160	0.317
Copper	0.572**	0.000	- 0.574**	0.000	- 0.484**	0.001	0.174	0.275	- 0.049	0.760

In our analysis, we observed a significant negative correlation between Zn and HOMA-IR (Fig. 4) in patients with insulin resistance. Additionally, a significant positive correlation between Zn and QUICK index was identified in the same patient group (Fig. 5).

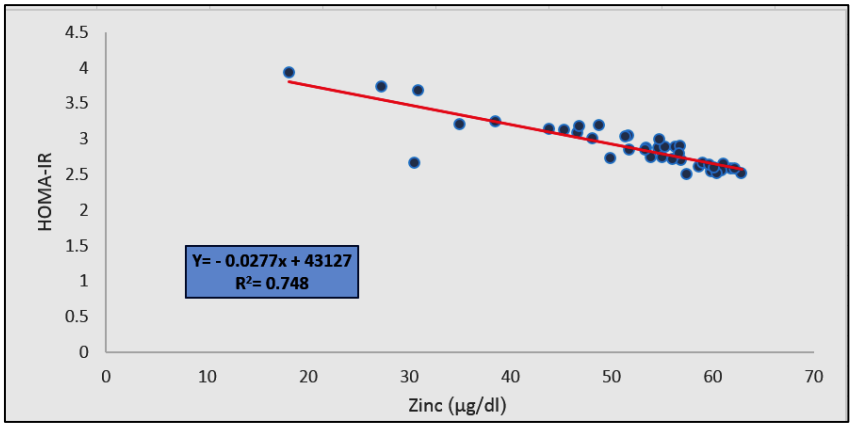

Figure 4. Correlation between serum Zn and HOMA-IR in Patients with İnsulin Resistance (r^2 = 0.784, *P*-value = 0.000)

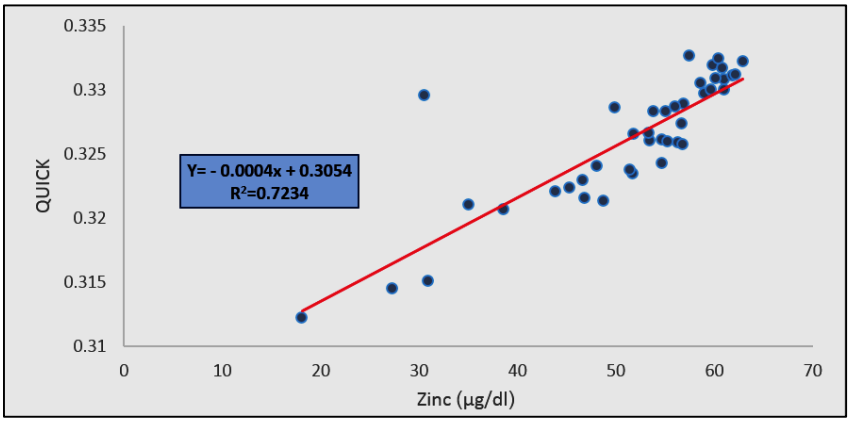

Figure 5. Correlation between serum Zn and QUICK in patients with insulin resistance (r^2 = 0.7234, *P*-value = 0.000).

Our investigation revealed a noteworthy negative correlation between Cu and QUICK (Fig. 6), as well as a significant positive correlation between Cu and HOMA-IR in patients with insulin resistance (Fig. 7).

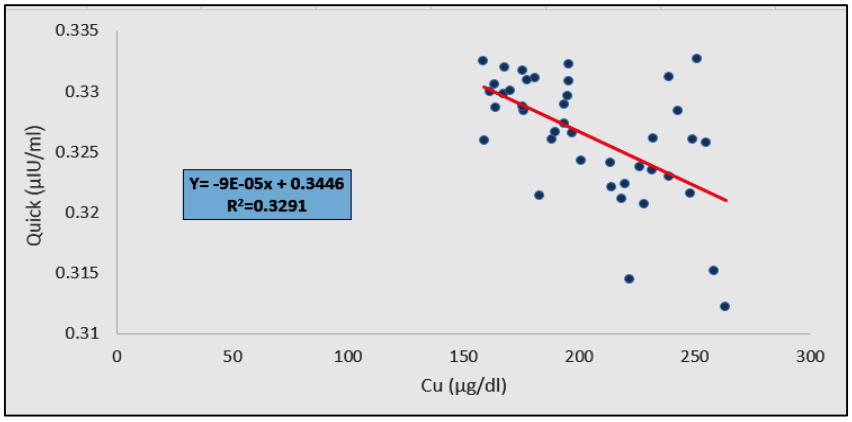

Figure 6. Correlation between serum Cu and QUICK in patients with insulin resistance (r^2 = 0.3291, *P*-value = 0.000).

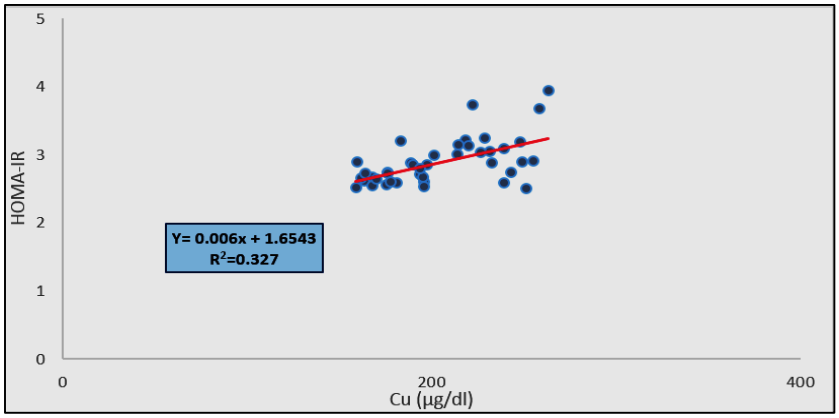

Figure 7. Correlation between serum Cu and HOMA-IR in Patients with İnsulin Resistance (r^2 = 0.327, *P*-value = 0.000).

2.4. The comparison of the study parameter values between Patients without Insulin Resistance Group and Control Group

Table 5 displays a comparison of the study parameter values between the Control Group and the Patients without Insulin Resistance Group. The study revealed significantly higher Cu levels in the Patients without Insulin Resistance Group compared to the Control Group, whereas Zn levels were markedly higher in the Control Group. These disparities in zinc and copper values between the Control Group and Patients without IR Group are attributed to their influential role in the context of PCOS independent of insulin resistance [31]. The insufficiency of zinc contributes to the pathogenesis of PCOS through causing defects in ovarian development [32], impairing the secretion of both FSH and LH [33], and acting as an anti-androgenic agent [34]. Conversely, excessive copper plays a role in the development of PCOS by affecting the secretion of adrenocorticotropic hormone and luteinizing hormone [35], reducing progesterone levels, and inhibiting the absorption of zinc, which is pertinent to the reproductive pathway [36,37].

A noteworthy observation was the significant inverse correlation identified between Cu and Zn in Patients with Insulin Resistance, as illustrated in (Fig. 8).

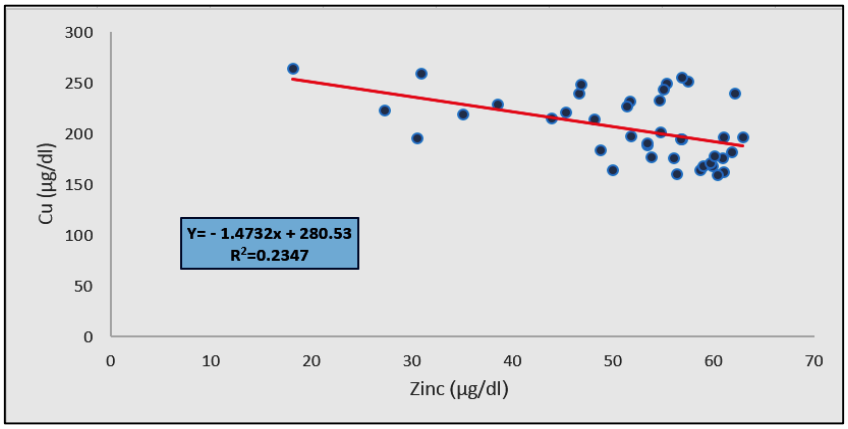

Figure 8. Correlation between serum Cu and serum Zn in patients with insulin resistance (r^2 = 0.2347, *P*-value = 0.001).

3. CONCLUSION

The study revealed a significant role for zinc and copper in the development of this syndrome, either through their association with insulin resistance or as isolated risk factors independent of insulin resistance. Consequently, we advocate for monitoring serum levels of zinc and copper and recommend maintaining them within the normal range, particularly for women with PCOS and women of childbearing age.

4. MATERIALS AND METHODS

This research was conducted at Al-Basil Hospital in Homs, Syria, spanning from June to September 2022, with each study participant providing informed written consent prior to participation.

4.1. Patients

Sixty-three (63) untreated adult female patients who were newly diagnosed with polycystic ovary syndrome were included in the study, along with 25 age-matched healthy subjects serving as the control group. The inclusion criteria stipulated that participants must be female, aged 18-43, and newly diagnosed with PCOS, without having commenced treatment. Exclusion criteria comprised individuals with diabetes, hyperprolactinemia, thyroid diseases, congenital adrenal hyperplasia, uterine cyst, breast cancer, epilepsy, Cushing's disease, and those who had used hormonal drugs, metformin, or supplements containing zinc and copper over specified time periods.

4.2. Samples

Venous blood samples were obtained from the enrolled patients after an overnight fasting period. The samples were collected from the cubital vein in sterile and dry plastic tubes and incubated in a water bath at 37°C for 30-45 minutes. Subsequently, the tubes underwent centrifugation at 3000 rpm for 10 minutes, leading to the separation of serum after clot formation. The serum was then divided into two parts, with the first part used for conducting biochemical tests (including fasting glucose, zinc, and copper) using a spectrophotometer. The second part was stored at -20°C for later measurement of fasting insulin via an ELISA device.

Insulin resistance was assessed using the homeostatic model assessment for insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI). An HOMA-IR value of ≥ 2.5 and a QUICKI value of ≤ 0.333 were considered indicative of insulin resistance. The HOMA-IR and QUICKI were calculated using their respective formulas.

$$HOMA\_IR = Glucose(mmol/L) * Insuline(mU/L)/22.5$$

$$QUICK1 = 1/\log Insulin\ (mU/mL) + \log Glucose\ \ (mg/dL)$$

4.3. Materials

In this study, various laboratory equipment was utilized, including 5 mL syringes, 5 mL dry tubes, yellow and blue micropipette heads, Eppendorf tubes, tube holders, micropipettes of different capacities, a centrifuge, and a water bath.

- Zınc monolıquıd: BIOREX / UNITED KINGDOM
- Copper (urıne/serum) colorımetrıc: BIOREX / UNITED KINGDOM
- Glucose: BIOSYSTEM / SPANISH
- Insulın ELISA: DIAMETRA / ITALY

4.4. Devices

The analysis of fasting blood glucose, zinc, and copper levels was conducted using a UV-visible spectrophotometer, while the analysis of fasting insulin hormone was performed using an ELISA device.

- ELISA: REBONIC
- U.V VIS spectrophotometer single beam, Simitronic

4.5. Statıstıcal Analysıs

Statistical analysis was performed using the Statistical

Package for the Social Sciences (SPSS) version 26.0 for Windows. Descriptive statistics were reported as mean ± standard deviation (SD). To compare means, Student's t-test was utilized. For examining binary correlations, Pearson's correlation test was employed, with the results presented as correlation coefficient (r) and P-value. Statistical significance was defined as $P < 0.05$.

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Conflict of interest statement: The authors declared no conflict of interest.

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العلاقة بين مستويات الزّنك والنّحاس ومقاومة الأنسولين لدى مريضات متلازمة المبيض متعدّد الكيسات في مدينة حمص

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ملخص

الهدف: دراسة العلاقة بين مستويات الزّنك والنّحاس ومقاومة الأنسولين الّتي هي الآليّة الإمراضيّة الرّئيسيّة لمتلازمة المبيض متعدّد الكيسات (PCOS)، ومقارنة مستويات المعادن مع الأشخاص الأصحّاء في حمص.

طريقة العمل: شمل البحث 63 مريضة تم تشخيصهن حديثاً بمتلازمة المبيض متعدّد الكيسات، وقبل أن تتم معالجتهن في مستشفى الباسل في حمص، سوريا، إلى جانب 25 امرأة سليمة من نفس العمر. تمَّ الحصول على عيّنات الدّم باستخدام الأنابيب الجافّة للقيام بمقايسة مستويات الزّنك والنّحاس والغلوكوز وهرمون الأنسولين. وفي وقت لاحق، تم حساب المؤشّرات التّالية HOMA-IR وQUICK.

النّتائج: في مجموعة المريضات، كانت مستويات الزّنك في الدّم أقل بشكل ملحوظ ($p = 0.000$)، وكانت مستويات النّحاس في الدّم أعلى بشكل ملحوظ ($p = 0.000$) مقارنة بالأشخاص الأصحّاء. في المريضات اللّواتي تعانين من مقاومة الأنسولين، كانت مستويات الزّنك في الدّم أقل بشكل ملحوظ ($p = 0.004$)، وكانت مستويات النّحاس في الدّم أعلى بشكل ملحوظ ($p = 0.000$) مقارنة بالمريضات اللّواتي لا تعانين من مقاومة الأنسولين. في المريضات اللّواتي لا تعانين من مقاومة الأنسولين مقارنة بالنّساء السّليمات، كانت مستويات الزّنك في الدّم أقل بشكل ملحوظ ($p = 0.000$)، وكانت مستويات النّحاس في الدّم أعلى بشكل ملحوظ ($p = 0.000$). وُجِد ارتباط إيجابي بين النّحاس و HOMA-IR ($r=0.572^{**}$) ($p=0.000$)، وارتباط سلبي بين الزّنك و HOMA-IR ($r=-0.865^{**}$) ($p=0.000$).

الخلاصة: إنَّ خلل مستويات الزّنك والنّحاس له آثار على تطوّر متلازمة المبيض متعدّد الكيسات، سواء ترافقت مع مقاومة الأنسولين أو تواجدت بشكل مستقل عنها.

الكلمات الدالة: متلازمة المبيض متعدّد الكيسات، الزّنك، النّحاس، مقاومة الأنسولين.

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Assessing Knowledge, Attitude, and Practice (KAP) towards Monkeypox among Healthcare Workers in JORDAN: A Cross-Sectional Survey

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ABSTRACT

Introduction: The zoonotic features and potential for transmission between animals and humans make the monkeypox (MPX) virus, a member of the orthopoxvirus family, a serious threat. Unfortunately, healthcare staff's lack of knowledge and readiness about MPX has made it harder to implement effective prevention and response plans. Our current understanding of the KAPs (Knowledge, Attitudes, and Practices) among Jordanian clinicians could benefit from additional research.

Methods: This cross-sectional study aimed to evaluate the MPX KAP among 300 healthcare workers (HCWs) in two private hospitals in the Irbid governorate of Jordan. We employed descriptive statistics, such as percentages and frequencies, as well as an independent sample t-test, one-way ANOVA, and multiple linear regression (enter method) for data analysis.

Results: Although differences existed between demographic groups, the study found that participants had an average level of MPX knowledge proficiency. Out of a total of 300 participants, 196 were female and 104 were male, with 52.7% in the 20–30 age bracket. Compared to their female counterparts, male respondents exhibited higher levels of knowledge, attitudes, and practices regarding MPX. Attitudes and practices towards MPX varied by age group and level of education, demonstrating how demographic factors impact these aspects of public health. Additionally, monkeypox attitudes were lower among women, those with a diploma degree, and those aged 31–40.

Conclusions: Healthcare staff require adequate training and continuous education to address their lack of knowledge and attitudes regarding MPX protection. Staying updated about new illnesses like MPX is crucial, as the COVID-19 pandemic demonstrated. To effectively tackle global health concerns, continuous learning and up-to-date knowledge are essential.

Keywords: monkeypox; Knowledge; Attitude; and Practice.

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1. INTRODUCTION

The global public health emergency designation has been assigned to the cross-border epidemic of human monkeypox (HMPX). According to Petersen et al. (2019), the orthopoxvirus group is where the MPX virus belongs. Public health professionals are debating whether the monkeypox virus poses a new threat due to the persistent pandemic it has caused (Centers for Disease Prevention and Control). Monkeypox (MPX) is a pathological condition that arises from infection with the MPX virus. Doty et al. (2017) have identified this ailment as a zoonotic viral illness, capable of transmission from animals to humans. Additionally, according to the World Health Organization (WHO), interpersonal contact can result in the transmission of the MPX virus. Khodakevich et al. (1987) were the first to identify the MPX virus in primates. However, it has also been found to spontaneously infect various other species, including rope squirrels, tree squirrels, Gambian pouched rats, and dormice. Lesions can range in severity from moderate to severe and may cause significant discomfort or irritation (Malik et al., 2023; Guarner et al., 2022). The origin of the animals remains undetermined; nonetheless, rats probably harbor the pathogen (Alshahrani & Algethami, 2022). Hunting involves engaging with both living and deceased animals, as well as consuming meat from wild animals or livestock, both of which have been identified as potential risk factors (Dada et al., 2022). The primary mode of transmission for MPX is through direct contact with nasal secretions, infected melanomas, or contaminated objects (Vaughan et al., 2020). The incubation period of MPX commonly ranges from 6 to 13 days, with a potential variation of 5 to 21 days (Reynolds et al., 2006). The symptoms typically manifest within a timeframe of fourteen to twenty-one days and are commonly self-limiting (Vaughan et al., 2020). The clinical manifestations of the disease resemble those of smallpox, albeit with a lesser degree of severity (Reynolds et al., 2006). According to Brown and Leggat (2016) and Schwartz et al. (2019), lymphadenopathy is a salient feature that distinguishes smallpox from MPX. Vaughan et al. (2020) stated that symptoms of monkeypox (MPX) include fever, headache, fatigue, swollen lymph nodes, back discomfort, muscle aches, and a rash. The efficacy of the smallpox vaccine in preventing monkeypox (MPX) was found to be 85%, according to studies conducted by Hammarlund et al. (2005) and Fine et al. (1988). The MVA-BN vaccine and tecovirimat, a specific therapeutic intervention for MPX, were granted approval by the Food and Drug Administration (FDA) in 2019 and 2022, respectively. Nevertheless, the accessibility of these preventive measures remains limited, resulting in a lack of widespread availability. Additionally, individuals below the age of 40 or 50 are no longer benefiting from the safeguards previously afforded by smallpox vaccination initiatives. Volkmann et al. (2021) and Borra et al. (2022) provided empirical evidence for the significance of preventive measures, early detection, and prompt management response, which Goyal et al. (2022) highlighted. Nevertheless, the World Health Organization (WHO) has identified a significant barrier to effectively preventing the re-emergence of MPX: the lack of understanding surrounding MPX, particularly among healthcare practitioners (Nadar et al., 2022). As a result, healthcare professionals need to know where monkeypox (MPX) is common, as this can make them more likely to get human MPX (Alshahrani & Algethami, 2022). Hence, the primary objective of this study was to:

1. investigate the knowledge, attitude, and practice (KAP) of healthcare personnel in two private hospitals in Irbid with regards to MPX.
2. assess whether there are statistically significant differences in knowledge, attitudes, and practices (KAP) among the participants based on their socio-demographic factors, namely gender, age, education level, and experience.
3. conduct an empirical investigation to examine the relationship between socio-demographic variables, including gender, age, education level, and

experience, and Knowledge, Attitudes, and Practices (KAP) scores.

2. METHODOLOGY

2.1. Study Design and Setting

A questionnaire was developed to assess the knowledge, attitudes, and practices (KAP) related to monkeypox among healthcare workers (HCWs) in the Irbid governorate of Jordan from April to July 2022. This study utilized a self-administered questionnaire specifically designed for this research objective. The questionnaire was distributed to participants in two well-established private hospitals for data collection.

2.2. Sample and Data Collection

The study included a diverse group of healthcare workers, encompassing physicians, nurses, paramedics, radiologists, and lab technicians, all of whom were employed at the designated facilities. Data collection from the two private hospitals was carried out using a convenience sampling methodology. Regarding the sample size, the study was confined to two privately owned hospitals, with efforts made to include all healthcare personnel employed within these facilities. The questionnaire was disseminated in the English language. A total of 500 questionnaires were distributed among the two hospitals, with a response rate of 300 questionnaires received by the research team.

2.3. Study Tool

The KAP (Knowledge, Attitudes, and Practices) questionnaire utilized in this study was adapted from instruments originally developed by Ahdab (2021) for cross-sectional investigations involving doctors in Syria. It was also derived from a previous study conducted by Zhong et al. (2020) that focused on COVID-19. Additionally, it included elements from H1N1 questionnaires, the severe acute respiratory syndrome (SARS) study by Lau et al. (2003), and theories like the health behavior principles examined by Rubin et al. (2014). The study employed two questionnaires to assess participants' knowledge and attitudes. The knowledge questionnaire comprised six multiple-choice questions, while the attitude questionnaire consisted of four multiple-choice questions. This survey included a set of seven multiple-choice questions designed for practice.

Two consultants with expertise in preventive medicine reviewed the initial version of the questionnaire, and their recommendations led to updates to the questions. A pilot test involving 18 healthcare workers evaluated the dependability of the questions. Based on the feedback received during the pilot test, the questionnaire was modified. The study questionnaires were subjected to an estimation of Cronbach's alpha, yielding an acceptable result of 0.74 (Taherdoost, 2016). The evaluation included an examination of socio-demographic factors, including age, gender, marital status, educational achievement, and professional experience. The survey required a time commitment of around 5 to 10 minutes for completion and was disseminated in the English language.

2.4. Data analysis

The data analysis was conducted using the Statistical Package for Social Sciences (SPSS, IBM, Chicago, IL, USA). Various statistical methods were employed, including descriptive statistics such as frequencies and percentages, the independent sample t-test to compare two groups, one-way ANOVA to compare more than two groups, and multiple regression analysis using the Enter technique.

3. RESULT

Three hundred fifty responses to the survey questionnaire were received; however, 50 had to be disregarded due to a high number of missing data. Thus, 300 participants were included in the final analysis. The age group between 20 and 30 comprised 48.7% (146) of the participants, and 64.7% (194) were females. Approximately 50.3% (151) of participants held a bachelor's degree, followed by 25.6% (77) with a diploma, and 24% (72) with a master's degree. Furthermore, the independent sample t-test indicated a significant difference

(P-value = 0.001) between male and female participants, with males demonstrating higher levels of knowledge about MPX (Mean = 3.44) compared to female participants (Mean = 2.97). Using one-way ANOVA, participants also differed statistically (P-value = 0.001) according to age groups. Participants aged 31-40 scored higher levels of knowledge about MPX compared to other age groups. More information is depicted in Table 1.

Table 1: Demographic characteristics of participants and the score of Monkeypox knowledge by socio-demographic variables

Characteristics		Number of participants (%)		Knowledge score (mean ± standard deviation)		P-value
Gender	**Male**	104	34.7%	3.44	1.21	**0.001**
	Female	196	65.3%	2.97	1.17	
Age	**20-30**	158	52.7%	2.89	1.13	**< 0.001**
	31-40	105	35.0%	3.72	1.14	
	41-50	37	12.3%	2.51	1.02	
Education	**Diploma**	116	38.7%	3.13	1.19	> 0.05
	Bachelor's degree	166	55.3%	3.15	1.24	
	Postgraduate	18	6.0%	3.06	1.00	
Experience	**One year and less**	77	25.7%	3.48	1.20	**< 0.05**
	One year to 5 years	95	31.7%	2.93	1.14	
	5 years to 10 years	44	14.7%	2.93	0.90	
	More than 10 years	84	28.0%	3.17	1.35	
Knowledge of Monkeypox score		300	100%	**3.14**	**1.20**	

No statistical differences (P-value > 0.05) in the level of knowledge about MPX were found among participants according to their education level. However, a statistical difference (P-value < 0.05) was found based on the length of experience. Participants with less than one year of experience had a higher level of knowledge (Mean = 3.48), followed by participants with more than ten years of experience (Mean = 3.17).

According to this questionnaire, healthcare workers have an average knowledge of MPX. The correct response rates for the six MPX knowledge questions ranged from 23.3% to 94% (Table 2). The mean knowledge score was 3.14 (SD: 1.20), indicating a correct answer percentage of 52.3% (3.14/6 * 100) on this knowledge test. Knowledge of MPX symptoms was highest (94%), while the perception of MPX severity was lowest (23.3%).

The overall attitude towards MPX was neutral. Most respondents believed that vaccination effectively controls the spread of MPX, with 71.0% agreeing (Table 2). Only 32.3% of the respondents expected MPX to spread in Jordan, whereas about 59.3% were confident that MPX would be controlled. Furthermore, most participants performed well regarding MPX prevention practices. Over 80% indicated that they avoid crowded places, wash their hands regularly, avoid shaking hands, practice better hygiene than before, and use hand disinfectant. However, wearing facemasks when in contact with someone showing symptoms received the lowest score among the practices performed by the participants.

Table 2: Summary of Questions for Knowledge, Attitudes, and Practices towards Monkeypox. Percentages represent the correct answers of Knowledge and the most common answers by healthcare workers of the attitudes and practices.

Knowledge
The main clinical symptoms of monkeypox are fever, headache, rash, and swollen lymph nodes (Yes, 94%)
Symptoms of monkeypox are similar to the common symptoms of smallpox (chickenpox) (Yes, 72.3%)
Monkeypox infection causes severe symptoms in all patients (No, 57.3%)
Persons with monkeypox can infect the virus others when a fever is not present (No, 39.7%)
Monkeypox infection causes serious disease (No, 23.3%)
Although there is no proven cure for monkeypox, the available treatments lead to recovery (No, 26%)
Attitudes
Do you think getting a vaccination is an effective way of preventing the spread of the disease (Yes, 71.0%)
Do you think the curfew is an effective way of preventing the spread of the disease (Yes, 63.0%)
Do you think that monkeypox will spread widely in Jordan (No, 54.7%)
Do you think that monkeypox will be successfully controlled (Yes, 59.3%)
Practices
Avoid crowded places (83.7%)
Avoid sex behaviors (kisses, touching) with someone who has the symptoms (84.3%)
Avoid shaking hands (84.3%)
Practice better hygiene than before (80.3%)
Use disinfectants (ethanol) with you are dealing with someone with symptoms (88.3%)
Wear facemasks when you contact someone with symptoms (72.0%)
Wash hands when you touch regularly (85.7%)

In Table 3, the independent sample t-test indicated a significant difference (P-value < 0.005) between male and female participants, with males scoring higher in attitudes towards MPX (Mean = 2.36) than female participants (Mean = 2.22). Using one-way ANOVA, participants differed statistically (P-value < 0.005) according to age groups. Participants aged 41-50 scored higher in attitudes towards MPX (Mean = 2.34) compared to other age groups. Additionally, statistical differences (P-value < 0.05) in attitudes towards MPX were found among participants according to their education level. Compared to diploma and bachelor's degree holders, participants with postgraduate degrees showed better attitudes towards MPX (Mean = 2.51). Moreover, no statistical difference (P-value > 0.05) was found in participants' attitudes towards MPX according to their experience. More information is depicted in Table 3.

The mean practice score was 5.80 (SD: 1.65), indicating a correct answer percentage of 82.85% (5.8/7 * 100) on this practice test. The independent sample t-test indicated a significant difference (P-value = 0.001) between male and female participants, with males scoring higher in practices towards MPX (Mean = 6.19) than female participants (Mean = 5.60). The one-way ANOVA test indicated no statistical differences (P-value < 0.005) according to age groups. Additionally, statistical differences (P-value < 0.05) in practices towards MPX were found among participants according to their education level. Compared to diploma and postgraduate holders, participants with bachelor's degrees showed

better practices towards MPX (Mean = 6.19). Furthermore, a significant statistical difference (P-value < 0.05) was found in participants' practices towards MPX according to their experience. Participants with 5-10 years of experience had a higher level of practice (Mean = 6.45). More information is shown in Table 4.

Table 3: Attitudes towards Monkeypox by socio-demographic variables

Characteristics		Attitudes score (mean ± standard deviation)		P-value
Gender	**Male**	2.36	0.39	**< 0.05**
	Female	2.22	0.49	
Age	**20-30**	2.31	0.41	**< 0.05**
	31-40	2.18	0.55	
	41-50	2.34	0.37	
Education	**Diploma**	2.19	0.49	**< 0.05**
	Bachelor's degree	2.30	0.43	
	Postgraduate	2.51	0.46	
Experience	**One year and less**	2.22	0.45	> 0.05
	One year to 5 years	2.32	0.44	
	5 years to 10 years	2.33	0.21	
	More than 10 years	2.22	0.57	
Attitudes towards Monkeypox		2.27	0.46	

Table 4: The score of practices towards Monkeypox by socio-demographic variables

Characteristics		Practices score (mean ± standard deviation)		P-value
Gender	**Male**	6.19	1.15	**0.001**
	Female	5.60	1.83	
Age	**20-30**	5.74	1.86	**> 0.05**
	31-40	5.74	1.44	
	41-50	6.24	1.14	
Education	**Diploma**	5.28	1.71	**< 0.001**
	Bachelor's degree	6.19	1.51	
	Postgraduate	5.61	1.61	
Experience	**One year and less**	5.75	1.66	**< 0.05**
	One year to 5 years	5.76	1.91	
	5 years to 10 years	6.45	1.07	
	More than 10 years	5.56	1.52	
Practices towards Monkeypox		5.80	1.65	

The results of multiple linear regression analyses of variables that scored significantly on KAP are depicted in Table 5. The results show that being female and having more than one year of experience are predictors of lower knowledge of Monkeypox compared to the reference groups (β: -0.303, P < 0.05; β: -0.494 to -1.024, P < 0.05, respectively). Furthermore, the results indicated that being female, being aged between 31-40, and having a diploma are predictors of a lower attitude towards Monkeypox compared to the reference groups (β: -0.171, P < 0.05; β: -0.202, P < 0.05; β: -0.249, P < 0.05, respectively). For practices towards Monkeypox, the results indicated that being female and having a diploma are predictors of lower practice compared to the reference groups (β: -0.171, P < 0.05; β: -0.202, P < 0.05).

Table 5: Results of multiple linear regression (Enter method) of sociodemographic factors associated with Monkeypox knowledge, attitude, and practice.

Variable / Knowledge	Coefficient (β)	Standard error	P-value	95.0% Confidence Interval for B	
				Lower Bound	Upper Bound
Gender (Male, reference)	1.000	1.000	1.000	1.000	1.000
Female	-0.303	0.138	**< 0.05**	-0.574	-0.032
Experience (1 year or less, reference)	1.000	1.000	1.000	1.000	1.000
One year to 5 years	-0.722	0.174	**< 0.001**	-1.065	-0.380
5 years to 10 years	-1.024	0.225	**< 0.001**	-1.466	-.582
More than 10 years	-0.494	0.241	**< 0.05**	-0.968	-0.020
Attitude					
Gender Male (reference)	1.000	1.000	1.000	1.000	1.000
Female	-0.171	0.058	**< 0.05**	-0.285	-0.057
Age (20-30, reference)	1.000	1.000	1.000	1.000	1.000
31-40	-0.202	0.073	**< 0.05**	-0.346	-0.058
Education Postgraduate(reference)	1.000	1.000	1.000	1.000	1.000
Diploma	-0.249	0.117	**< 0.05**	-0.480	-0.018
Practices					
Gender (Male, reference)	1.000	1.000	1.000	1.000	1.000
Female	-0.089	0.042	**< 0.05**	-0.172	-0.007
Education (Postgraduate, reference)	1.000	1.000	1.000	1.000	1.000
Diploma	-0.067	0.085	**< 0.05**	-0.234	0.100

4. .DISCUSSION

It is noteworthy to acknowledge that there is a significant lack of awareness regarding monkeypox (MPX) infection among the general public, medical practitioners, and policymakers in low-income countries (Khan et al., 2022). The issue of conspiracy theories surrounding new virus infections has garnered attention due to the ongoing outbreaks in multiple nations globally (Sallam et al., 2022).

In the final analysis, a total of 300 participants were included in the study. The largest demographic group consisted of individuals aged 20 to 30, representing 48.7% (146 individuals) of the overall sample. Within this age bracket, females comprised 64.7% (194 individuals) of the participants. The study findings revealed that male participants demonstrated a higher

level of expertise in MPX, with a mean score of 3.44, compared to female participants, who had a mean score of 2.97. These findings were consistent with previous studies conducted by Youssef et al. (2023) and Sallam et al. (2022).

However, the study did not identify significant disparities in awareness levels of MPX across individuals with different educational backgrounds. The survey findings indicated that healthcare practitioners possess a moderate level of understanding regarding monkeypox, confirming the results presented in Youssef et al. (2023). An inverse relationship was found between advanced age and high levels of knowledge about the subject matter. The findings suggest no significant disparities in MPX knowledge among participants based on their education level. This contradicts the conclusions drawn by previous studies (Youssef et al., 2023; Malaeb et al., 2023; Kumar et al., 2022), which suggested that individuals with a postgraduate degree tend to possess greater knowledge of MPX.

Additionally, it was observed that healthcare workers with less than one year of experience demonstrated a higher level of proficiency in knowledge compared to their counterparts with greater experience. Importantly, the study found a high level of knowledge regarding MPX symptoms, reported at 94%. Interestingly, those with less than one year of experience had a higher level of knowledge in this regard.

A comprehensive understanding of the MPX virus and its associated ailments is crucial in helping communities enhance their preparedness and effectively manage a potential MPX epidemic. The study investigated the knowledge, attitudes, and practices (KAP) related to MPX, with a specific focus on identifying demographic factors associated with these attributes. These findings have the potential to inform public health officials and healthcare workers in targeting interventions aimed at preventing and educating about MPX during potential future outbreaks, consistent with Youssef et al. (2023), Maqableh et al. (2024), and Kumar et al. (2022).

Participants noted that a significant portion of respondents had a reasonable level of general knowledge about monkeypox (MPX). This finding contradicts the results of Bates and Grijalva (2022), who reported very low levels of MPX knowledge among participants. The study's findings on attitudes and practices surrounding MPX among healthcare workers (HCWs) indicate a diverse landscape. Generally, attitudes towards MPX were characterized by a moderate level of neutrality. The study found that a majority of participants (71.0%) believed in the effectiveness of vaccination to prevent the spread of MPX. However, this percentage is slightly lower than that reported by Alshahrani et al. (2022), who found a confidence level of 78.6% in the Saudi Ministry of Health's ability to manage MPX locally.

Furthermore, 46% of participants believed that MPX could spread in Jordan. In contrast, Alshahrani et al. (2022) reported that 64.6% of their study sample believed MPX could easily spread to Saudi Arabia. Regarding variations among healthcare workers based on socio-demographic characteristics, there are projections of a third wave of MPX transmission in Jordan. Nonetheless, a substantial proportion of individuals (59.3%) expressed confidence in their ability to manage MPX.

Attitudes among healthcare workers varied based on socio-demographic factors such as gender, age, and education level. While Malaeb et al. (2023) partially contradicted these findings by suggesting that attitudes do not differ based on gender (consistent with Kumar et al., 2022 and Al-Taani et al., 2023), they observed significant variations in attitudes based on education level.

The majority of participants demonstrated positive behaviors towards preventive measures, with over 80% adhering to guidelines such as avoiding crowded areas, regular handwashing, and maintaining improved hygiene. Notably, the least commonly implemented

measure was the use of face masks when in contact with symptomatic individuals. Individuals with postgraduate degrees tended to hold more positive attitudes, while those with bachelor's degrees tended to exhibit more favorable habits (Bates et al., 2022).

According to Lulli et al. (2022), there was a negative correlation observed between being female, having over one year of experience, and lower levels of MPX knowledge. Similarly, being female, aged 31–40, or holding a certificate degree was associated with weaker sentiments towards MPX. Likewise, females with a diploma tended to engage less in MPX-related practices.

The study's results underscore the importance of tailored interventions focusing on specific demographic groups to enhance healthcare workers' understanding, attitudes, and practices related to MPX. Considering gender differences, levels of experience, and educational backgrounds can significantly influence the knowledge and implementation of MPX management techniques within this professional group.

5. CONCLUSION

Cross-sectional studies consistently indicate that healthcare workers have insufficient knowledge, attitudes, and practices (KAP) regarding protection from monkeypox virus (MPXV). Proper training and continuous, thorough education on emerging diseases are imperative. Evaluating information assumes critical importance, especially in light of previous epidemics and pandemics like COVID-19, which underscore the need for interconnected and specialized knowledge. As global interconnectedness increases, it becomes crucial for individuals to remain informed and educated about emerging diseases such as MERS-CoV, including understanding necessary precautions and best practices to prevent their spread. Lessons learned from past outbreaks like COVID-19 highlight the necessity of continuous learning and updated knowledge to effectively combat global health threats.

6. LIMITATION

Limitations of the study include potential sampling bias due to limited hospital representation, missing data affecting sample inclusivity, and reliance on self-reported responses susceptible to social desirability bias. The study's scope focused on specific questions about monkeypox (MPX), potentially overlooking broader aspects and requiring more longitudinal insights. It did not consider potential confounding factors, which limits comprehensive understanding. Additionally, the study did not account for regional variations in monkeypox prevalence, thereby potentially limiting its generalizability to other geographic areas.

The cross-sectional design of the study prevents establishing causal relationships between variables and only offers a snapshot of the situation at a specific point in time. Furthermore, the findings may not be applicable to other populations or settings due to the specific context in which the data were collected. Additionally, reliance on self-reported responses may introduce recall bias, as participants may not accurately remember or report their experiences with monkeypox.

7. DECLARATIONS

The authors provide no declaration of a conflict of interest

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تقييم المعرفة والمواقف والممارسات تجاه جدري القرود بين العاملين في مجال الرعاية الصحية في الأردن: مسح مقطعي

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ملخص

مقدمة: إن الخصائص الحيوانية المنشأ وإمكانية انتقالها بين الحيوانات والبشر تجعل من فيروس جدري القرود , وهو عضو في عائلة فيروسات الجدري الأورثوبوكس، تهديدًا خطيرًا. ولسوء الحظ، فإن افتقار موظفي الرعاية الصحية إلى المعرفة والاستعداد بشأن فيروس جدري القرود جعل من الصعب وضع خطط فعالة للوقاية والاستجابة. إن فهمنا الحالي لـ فيروس جدري القرود في مجموعات الأطباء الأردنيين يمكن أن يستفيد من الأبحاث الإضافية.

الطرق: كان الغرض من هذه الدراسة المقطعية هو تقييم المعرفة، المواقف والممارسة لفيروس جدري القرود،.بين 300 من العاملين في مجال الرعاية الصحية الذين يعملون في مستشفيين خاصين في محافظة إربد في الأردن. بالنسبة لهذه البيانات، استخدمنا إحصائيات وصفية مثل النسب المئوية والتكرارات، بالإضافة إلى اختبار t لعينة مستقلة، وANOVA أحادي الاتجاه، والانحدار الخطي المتعدد (طريقة الإدخال).

النتائج: على الرغم من وجود اختلافات بين المجموعات الديموغرافية، فقد وجدت الدراسة أن المشاركين لديهم مستوى متوسط من إتقان المعرفة بفيروس جدري القرود. ومن بين إجمالي 300 مشارك، كان 196 من الإناث و104 من الذكور. وكان 52.7% منهم في الفئة العمرية 20–30 عامًا. بالمقارنة مع نظرائهم من الإناث، أظهر المشاركون الذكور مستويات أعلى من المعرفة والمواقف والممارسات فيما يتعلق بفيروس جدري القرود. تختلف المواقف والممارسات تجاه فيروس جدري القرود حسب الفئة العمرية ومستوى التعليم، مما يوضح كيفية تأثير العوامل الديموغرافية على هذه الجوانب من الصحة العامة. بالإضافة إلى ذلك، كانت المواقف تجاه جدري القرود أقل بين النساء، والحاصلات على درجة الدبلوم، وأولئك الذين تتراوح أعمارهم بين 31 و40 عامًا.

الاستنتاجات: من الواضح أن موظفي الرعاية الصحية يحتاجون إلى تدريب مناسب وتعليم مستمر لمعالجة افتقارهم إلى المعرفة والمواقف فيما يتعلق بحماية فيروس جدري القرود. يعد البقاء على اطلاع بشأن الأمراض الجديدة مثل فيروس جدري القرود أمرًا بالغ الأهمية، كما أظهر جائحة كوفيد–19. ومن أجل معالجة المخاوف الصحية العالمية بشكل فعال، يجب على المرء أن يتعلم باستمرار وأن يكون لديه أحدث المعرفة.

الكلمات الدالة: جدري القرود، المعرفة، الموقف، والممارسة.

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Infrared Microscopy: A Multidisciplinary Review of Techniques, Applications, and Ethical Dimensions

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ABSTRACT

Infrared microscopy has become a significant analytical technique with a transformative impact on various scientific disciplines. This review examines its applications in biomedical research, materials science, environmental monitoring, and art conservation. The non-invasive and label-free technique has revolutionized disease diagnostics, drug discovery, and tissue engineering by providing comprehensive molecular and cellular insights. In materials science, it has significantly advanced understanding of microstructure and material properties, facilitating the development of novel materials. In environmental monitoring, infrared microscopy plays a crucial role in assessing microplastics and atmospheric pollutants, supporting environmental protection efforts. In art preservation, the technique offers valuable insights into the composition and deterioration of historical artworks. Recent advancements in sensor technology, particularly InGaAs and graphene-based detectors, coupled with artificial intelligence and machine learning, have greatly enhanced image analysis capabilities. The review identifies key challenges such as surpassing the diffraction limit and interpreting complex data. Ethical concerns, including data privacy and equitable access to technology, are also emphasized. Infrared microscopy remains a vital tool for advancing scientific knowledge and practical applications. Its impact is poised to expand with future technological developments, contingent upon addressing both technological challenges and ethical considerations.

Keywords: Infrared Microscopy Techniques; Sample Preparation; Multidisciplinary Applications; Ethical Considerations; Machine Learning and AI Techniques.

INTRODUCTION

Introduction to Infrared Imaging

Infrared (IR) imaging offers a unique opportunity to explore hidden aspects of the universe beyond human vision. In recent years, this advanced technology has made significant progress, providing valuable insights across biomedical science, environmental research, art conservation, and industrial applications. This article explores the fundamental principles of infrared imaging to give readers a comprehensive understanding of this intriguing field, as depicted in Figure 1.

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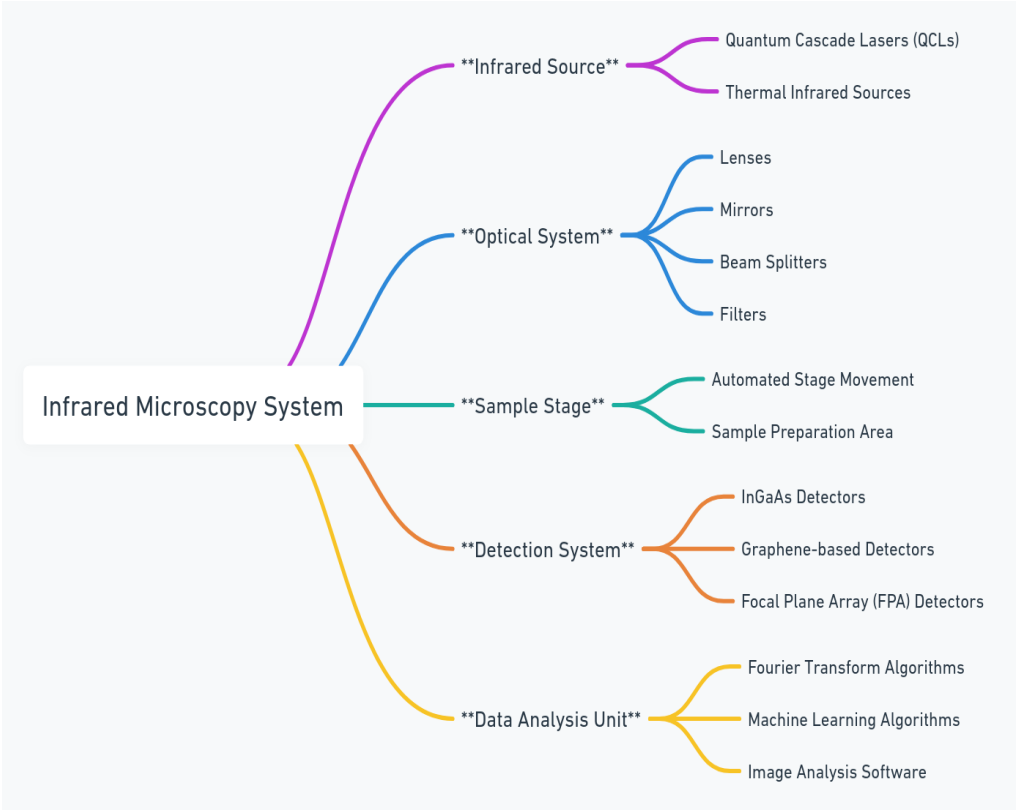

Figure 1 The essential elements of an infrared microscopy system. The diagram illustrates the main components of the system, such as the Infrared Source, Optical System, Sample Stage, Detection System, and Data Analysis Unit, along with their respective subcomponents.

The potential for significant advancements in both science and technology makes infrared imaging an area of great interest. The electromagnetic spectrum encompasses a wide range of wavelengths, from visible light to ultraviolet radiation and infrared rays. Specifically, the infrared portion extends from 700 nanometers (nm) to 1 millimeter (mm) [1]. Infrared radiation is categorized into three groups: near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR) [2], each with unique characteristics offering specific advantages in various fields. IR imaging systems detect infrared radiation emitted or reflected by objects and convert it into electrical signals [3]. These signals are processed to generate intricate thermal maps that reveal details beyond human vision [4]. Traditional IR imaging techniques commonly use specialized sensors and thermal cameras [5]. The ability to convert these signals into visual images provides significant insights into the environment, revealing previously unidentified natural phenomena and enabling new avenues of exploration and understanding. The integration of conventional microscopy with infrared spectroscopy in infrared microscopy has transformed scientific research by providing unparalleled spatial resolution and chemical specificity at the microscale. This non-invasive, contactless technology offers detailed information about materials without causing harm or alteration [6]. Its versatility allows adaptation to various environments and experimental setups, fostering new opportunities for

exploration. This review examines cutting-edge techniques that have propelled infrared microscopy to the forefront of discovery and explores the applications enabled by these advances. It also addresses the ethical implications and future prospects of infrared imaging technologies in this rapidly progressing field. By unveiling invisible phenomena in science, these methodologies enhance our understanding and revolutionize our approach to research and innovation.

The Emergence of Infrared Microscopy: A Brief History

The origins of infrared microscopy can be attributed to Sir Frederick William Herschel, a British astronomer and musician. In 1800, Herschel made the remarkable discovery of previously unseen radiation within the electromagnetic spectrum [7]. His experiments revealed a previously unidentified energy source, sparking further investigation in this emerging area of research. In the early 20th century, significant progress was made in thermal detection, specifically with thermopiles and bolometers. These advances paved the way for initial endeavors in visualizing infrared radiation [8]. The development of Fourier transform infrared (FTIR) spectroscopy in the mid-20th century marked a significant milestone in the advancement of FTIR microscopy. This technique allowed researchers to achieve unprecedented precision in probing chemical composition and molecular structure. Herschel's serendipitous discovery paved the way for significant technological advances, particularly in infrared microscopy. As a result, we are now in a modern era characterized by rapid technological progress and a wide range of applications for this microscopy technique.

The 21st century has brought about a new era in infrared microscopy. Recent advancements in techniques and technologies have significantly expanded the boundaries of achievable outcomes, enabling spatial resolutions and acquisition speeds that were previously unattainable. The integration of quantum cascade lasers (QCLs) and near-field microscopes has transformed the field, leveraging advanced computational techniques and machine learning algorithms to provide an extraordinary scientific experience [9].

Principles and Fundamentals of Infrared Microscopy

Infrared microscopy has revolutionized scientific exploration by enabling investigation into the previously unobservable realm of infrared radiation. To fully grasp the capabilities and possibilities of this phenomenon, it is crucial to understand the underlying principles that govern its functioning. Infrared microscopy operates by identifying unique absorption patterns exhibited by molecules within a sample, thereby providing insights into their chemical composition and structural characteristics [10]. A comprehensive understanding of optical microscopy and infrared spectroscopy is necessary to comprehend this process. Spectroscopy reveals different absorption patterns, known as molecular "fingerprints," arising from the unique energy levels present in different molecules [11]. Through spectral analysis, researchers gain valuable insights into the molecular composition of sample materials and develop an accurate understanding of their characteristics.

Optical microscopes utilize visible light and magnifying lenses to produce enlarged images. However, their resolution is constrained by diffraction, limiting them to approximately half the wavelength of the light used, often falling within the range of invisible light [12]. Specialized optics can overcome this limitation, enabling higher-resolution imaging and more advanced chemical analysis capabilities associated with infrared microscopy techniques. Integration of infrared microscopy has significantly transformed microscale imaging and chemical analysis by combining two robust techniques into a unified platform [13]. This approach utilizes modern Fourier transform infrared (FTIR) spectroscopy to provide enhanced spectral resolution, improved signal-to-noise ratio, and faster data acquisition. The tunable nature of Quantum Cascade Laser (QCL)-based systems and near-field infrared microscopy has led to significant advancements in achieving exceptional levels of detail and specificity.

Researchers employ various sampling methods, such as transmission, reflection, or attenuated total reflectance

(ATR), to analyze different sample types under varying conditions. Advanced computational algorithms, including multivariate analysis and machine learning, efficiently perform complex data analyses and extract valuable information rapidly [14]. This text provides an in-depth overview of the fundamentals of infrared imaging technology, potential applications (as shown in Table 1), and its transformative impact in scientific research.

Table 1. A comparative analysis of various infrared microscopy techniques, focusing on their scientific principles, advantages, limitations, and key references.

Technique	Principle	Advantages	Limitations	Reference
Raman Infrared Microscopy	Inelastic scattering of light by sample molecules; shift in frequency provides molecular information	Label-free; Non-destructive; Complementary to IR microscopy	Limited sensitivity; Fluorescence interference	[15]
Thermal Infrared Microscopy	IR detection of temperature differences in the sample due to its thermal properties	Non-contact; Non-destructive; Useful for material analysis	Limited to samples with temperature variations	[16, 17]
Fourier Transform Infrared (FTIR) Microscopy	Interferometry and Fourier Transform to obtain IR spectra	High resolution; Rapid data acquisition; High sensitivity	Requires precise alignment and calibration	[18]
Quantum Cascade Laser-based Infrared Microscopy	Tunable laser sources for the IR spectral region; narrow linewidth	High spatial and spectral resolution; Fast acquisition	Limited to a specific wavelength range	[19]
Near-field Infrared Microscopy	Near-field scanning optical microscopy with IR light source	Sub-wavelength spatial resolution; Non-destructive	complex setup; Slow imaging process	[20]
InGaAs Detector-based Microscopy	Indium Gallium Arsenide detectors for NIR region	High sensitivity; Fast response time	Limited to the NIR range	[21]
Graphene-based Detectors	Graphene's unique electronic properties for IR detection	Broadband detection; Ultrafast response; High sensitivity	Requires advanced fabrication techniques	[22]
Transmission Infrared Microscopy	The transmitted IR light passes through the sample and is detected	Useful for thin samples; Good for biological applications	Limited to transparent samples	[23]
Reflection Infrared Microscopy	Reflected IR light from the sample surface is detected	Suitable for opaque samples; Non-destructive	Limited to surface analysis; Possible specular reflections	[24]
Attenuated Total Reflection (ATR) Infrared Microscopy	Sample in contact with ATR crystal; evanescent wave interacts with the sample	Minimal sample preparation; Suitable for most samples	Limited penetration depth; Requires good sample contact	[25]
Hyperspectral Infrared Microscopy	Combines IR microscopy with hyperspectral imaging for detailed spectral and spatial information	High spectral and spatial resolution; Rich data sets	Longer acquisition times; Requires advanced data processing	[26]
Time-resolved Infrared Microscopy	Time-gated detection of transient IR signals	Studying fast chemical reactions or transient phenomena	Requires specialized equipment; Limited temporal resolution	
Polarization-sensitive Infrared Microscopy	Analyzing Polarization-Dependent Response of the sample to IR light	Provides insight into the sample's anisotropy and orientation	Limited to samples with anisotropic properties	[27]

Fourier Transform Infrared (FTIR) Microscopy: Advancements in Spectral Imaging and Analytical Capabilities

FTIR microscopy transforms scientific observation of samples by providing exceptional chemical specificity at the microscale. FTIR combines advanced spectral analysis with optical microscopy to efficiently capture high-resolution infrared spectra in significantly less time compared to conventional techniques. The fundamental aspect of this method is Fourier transform; a mathematical technique used to analyze interferograms acquired from an interferometer. It effectively converts these interferograms into high-resolution infrared absorption spectra.

FTIR microscopy has revolutionized interdisciplinary research, spanning materials science and biomedical imaging. It offers notable advantages over alternative spectroscopic techniques, primarily due to its swift data acquisition and remarkable signal-to-noise ratio achieved through Fellgett's Advantage or multiplex advantage [28]. To enhance these benefits, advanced sampling modes such as transmission, reflection, and attenuated total reflectance (ATR) have been developed. These modes are suitable for various sample types and experimental conditions. The ATR mode, in particular, enables surface probing of challenging samples that are not suitable for transmission measurements due to the internal reflection element [29].

Incorporation of focal plane array (FPA) detectors into Fourier transform infrared (FTIR) microscopy systems has significantly enhanced the technique's capabilities [5]. These detectors enable researchers to efficiently generate high-resolution chemical maps over large areas by collecting extensive spectral data from thousands of spatial points simultaneously. With contemporary computational techniques and advanced hardware advancements like FPA detectors [19], FTIR microscopy is leading the field of spectral imaging, enabling scientists to address complex issues and gain unprecedented insights.

Multivariate analysis techniques, such as principal component analysis (PCA) and partial least squares (PLS) regression, effectively extract valuable information from complex datasets. Additionally, machine learning algorithms proficiently identify subtle features and construct predictive models using FTIR data [30].

Comparative Analysis of Mapping and Imaging Modalities in Fourier Transform Infrared (FT-IR) Microscopy

Fourier Transform Infrared (FT-IR) microscopy utilizes two main modalities: mapping and imaging. Although they are often used interchangeably, these terms refer to separate techniques in the acquisition and visualization of spectral data [31]. Mapping in FT-IR involves collecting spectra at defined points across the sample [32]. Typically, the stage holding the sample moves in an X-Y grid pattern, and a spectrum is recorded at each point. This results in a series of individual spectra, which can be processed and compiled into a map representing specific chemical information. FT-IR mapping is highly versatile, allowing for high-resolution analysis; however, it can be time-consuming, especially for large areas [33].

In contrast, FT-IR imaging involves the simultaneous collection of spectral data over a wide area of the sample [34]. Using a focal plane array (FPA) detector, it captures spatial information in two dimensions along with the spectral dimension [35]. Therefore, FT-IR imaging is often faster and more suited for analyzing larger samples or providing a general overview of the sample composition. However, it may have lower spectral resolution compared to mapping [36]. In practice, the choice between mapping and imaging depends on the specific requirements of the analysis. Mapping is preferred when pinpoint spectral accuracy and high spatial resolution are paramount, such as in the analysis of microstructures or small inclusions within materials. Imaging, on the other hand, may be chosen for a more comprehensive, rapid assessment of larger areas or when contextual information is crucial.

In summary, FT-IR mapping excels at detailed, high-resolution chemical characterization at specific points [37], while FT-IR imaging provides a broader view by

simultaneously capturing spectral and spatial information across an extended area [38]. Both modalities are invaluable tools in infrared microscopy, and their judicious application can yield rich insights into the molecular and structural attributes of samples across diverse fields.

Quantum cascade laser-based infrared microscopy

Quantum Cascade Lasers (QCLs) have significantly transformed infrared microscopy by introducing unparalleled performance and functionality. Leveraging intersubband transitions in quantum wells to produce tunable, coherent radiation across mid- and far-infrared regions, these semiconductor lasers offer numerous advantages for scientific research [39]. QCL-based infrared microscopy enables rapid data acquisition with improved signal-to-noise ratios, allowing real-time, in-situ measurements [40]. This capability is supported by their broad tunability and high spectral brightness, surpassing existing technologies.

Advanced detection techniques such as Focal Plane Arrays (FPAs) benefit from QCLs' increased brightness, providing researchers with hyperspectral images and exceptional spatial resolution in record times [41]. One notable advancement is Quantum Cascade Laser-based Near-Field Scanning Optical Microscopy (QCL NSOM), combining QCLs and near-field scanning optical microscopy (NSOM). This technique surpasses the diffraction limit, achieving resolutions in the tens of nanometers range. It opens novel opportunities in nanoscale material characterization, particularly in plasmonics, phonon polaritons, and molecular vibrations, offering unprecedented detail.

QCL-based infrared microscopy has significantly impacted various scientific disciplines, including materials science, environmental monitoring, and biomedical imaging [43, 44]. Its broad applications facilitate the investigation of semiconductor materials and precise analysis of defects in two-dimensional materials, as well as the precise mapping of chemical composition within biological tissues.

Near-field infrared microscopy

Consider a hypothetical scenario where the diffraction limit no longer restricts observation, enabling us to explore the intricate and enigmatic nanoscale. Near-field infrared microscopy (NFIM) has overcome this barrier, providing unparalleled insights into nanomaterials [45]. This innovative method holds immense potential for scientific investigation, inspiring wonder with its capability to reveal previously inconceivable information. The fundamental concept integrates near-field scanning optical microscopy (NSOM) principles with infrared spectroscopy, generating evanescent fields that are probed using specific methods such as aperture probes or scattering-type scanning near-field optical microscopes (s-SNOM). These fields are then transformed into propagating waves, producing high-resolution images with spatial resolutions as low as tens of nanometers.

NFIM's innovative technology offers unprecedented access to data on chemical composition, structural characteristics, and vibrational information at the nanoscale [46], providing significant insights into material behavior in nano-environments. This capability allows researchers to understand the dynamic nature of our universe and discover transformative applications. The term "near-field" refers to the region in close proximity to an object or source of interest.

Infrared microscopy has profoundly influenced various fields, including two-dimensional materials, plasmonic nanostructures, the study of biological systems, and molecular assemblies [47, 48]. The field continues to advance with the introduction of quantum cascade lasers (QCLs), which offer enhanced brightness and rapid tunability [49], along with novel probe designs and fabrication methods enabling higher-resolution imaging and simultaneous measurement of multiple sample properties [50].

Advancements in Sensor Technologies: A Comparative Study of InGaAs and Graphene-Based Detectors in Future Applications

InGaAs detectors are widely recognized as the preferred choice for near-infrared spectroscopy and imaging due to their high quantum efficiency, minimal dark current, and excellent temperature stability [51]. The introduction of graphene-based sensors has significantly

enhanced the performance and sensitivity of spectral imaging, sparking a revolution in this field. This article aims to comprehensively explain InGaAs and graphene-based detectors, aiming to pique curiosity about the potential capabilities of these innovative sensors.

Advancements such as the adoption of large-format focal plane arrays (FPAs) and high-speed cameras based on InGaAs technology have expanded the application range of these detectors in fields such as telecommunications, environmental monitoring, and biomedical imaging [52]. Graphene-based sensors are particularly transformative in infrared detection, surpassing the capabilities of InGaAs detectors. Graphene, composed of a single layer of carbon atoms arranged in a hexagonal lattice, exhibits unique electronic and optical properties that make it highly suitable for advanced sensor applications [53, 54].

Graphene-based detectors offer several advantages over traditional InGaAs detectors, including a broader spectral response, enhanced responsivity, and rapid response times. Moreover, their compatibility with Complementary Metal-Oxide-Semiconductor (CMOS) fabrication processes enables cost-effective production of sensor arrays on a large scale [55]!

Exploiting Infrared Imaging CCD Cameras: Transforming Sensitivity and Resolution

The integration of charge-coupled device (CCD) cameras has revolutionized infrared (IR) imaging, enabling the capture of high-resolution images across the infrared spectrum [56]. These cameras convert light into electronic signals interpretable as pictures [57]. CCDs designed for infrared imaging exhibit remarkable sensitivity to IR radiation [58], facilitated by specialized sensors and cooling mechanisms that enhance detection accuracy of faint IR signals. Enhanced spatial resolution is another significant benefit [59]; each photodetector corresponds to a pixel on the image, with higher-end models featuring densely packed arrays of detectors for sharper visuals, making them particularly suitable for detailed material science studies at microscopic levels.

Excellent quantum efficiency further enhances their advantages [60, 61]; photons are efficiently converted into electrons, resulting in images with enhanced contrast and dynamic range, attributable to reduced internal camera noise levels [62]. Moreover, these devices can integrate seamlessly with various optical components or filters to meet specific application needs; for instance, biomedical imaging may require specific wavelengths to enhance visibility of certain cellular components over others [63].

Multispectral and Hyperspectral Imaging

Multispectral photography captures images using various spectral bands, enabling simultaneous analysis across multiple wavelengths and providing an enhanced perspective on reality [64]. This technology finds extensive application in remote sensing for agriculture, environmental monitoring, and cultural heritage conservation efforts. Multispectral imagery allows researchers to uncover crucial information regarding materials' composition, structures, and functional properties.

Hyperspectral imaging offers precise spectral signatures from a wide range of contiguous bands [65]. Advances in sensor technologies such as InGaAs and graphene detectors have led to the development of compact yet highly efficient imaging systems capable of capturing detailed spectral information at high resolutions across a broad range of wavelengths. Machine learning algorithms, along with deep learning techniques, have expanded capabilities in the analysis of multispectral and hyperspectral data [66].

Computational Approaches in Image Analysis: Integrating Machine Learning and Artificial Intelligence for Advanced Data Interpretation

The application of advanced artificial intelligence (AI) and machine learning techniques has significantly transformed the field of infrared imaging through the analysis of multispectral and hyperspectral images. Computational methods such as k-means clustering, hierarchical clustering, support vector machines, and

random forests provide efficient means to extract valuable insights from complex image data [67, 68].

Deep learning, a subfield of machine learning, enables the creation of sophisticated artificial neural networks capable of capturing intricate patterns and abstractions in datasets. Convolutional neural networks (CNNs), in particular, have gained popularity and demonstrated efficacy in various image analysis tasks, including object recognition, segmentation, and classification [69, 70]. Advanced neural network models like Residual Networks (ResNets) and U-Nets have significantly advanced infrared image processing [71, 72]. ResNets address the issue of vanishing gradients through deep layers and skip connections, enabling comprehensive analysis of intricate image features [73]. U-Nets have shown exceptional performance in segmenting infrared images, crucial for applications such as thermal fault detection, due to their unique encoder-decoder architecture [74].

The integration of multispectral imaging techniques and advanced computational methods, such as AI and ML, has expanded the capabilities of infrared imaging. This combination allows for the detection and analysis of complex spectral signatures and subtle variations in material composition or functional properties [27].

Domain adaptation techniques within transfer learning frameworks have become pivotal in IR imaging, addressing challenges posed by domain shift when models trained on visible spectrum images are adapted to perform accurately on infrared spectrum images. This recalibration is crucial in applications like cross-spectral medical diagnostics, where models trained on extensive RGB image datasets need adjustments for interpreting IR images [75]. For instance, models originally trained on the ImageNet dataset have been successfully adapted for thermal image analysis [76, 77], enhancing early detection of skin diseases and reducing reliance on extensive thermal datasets.

Generative Adversarial Networks (GANs) have significantly improved the quality of infrared images. Super-resolution GANs (SRGANs) are employed to enhance the resolution of low-resolution infrared images, a common challenge in thermal imaging due to sensor limitations [78, 79]. SRGANs use adversarial training to generate high-resolution images that retain crucial thermal details essential for accurate analysis [80]. This technology has been applied extensively in urban thermal imaging [81, 82], making significant contributions to heat mapping and energy efficiency research.

The emerging field of infrared (IR) imaging holds immense potential for advancements across various disciplines, as highlighted in Table 2, including environmental monitoring and medical diagnostics.

Elucidating Molecular Structures via Advanced Sample Preparation Techniques in Infrared Microscopy

Sample preparation is crucial for successful infrared microscopy, ensuring optimal imaging conditions and maximizing data quality [91]. To achieve maximum light transmission through the sample, thin sections must be created using techniques such as microtoming, cryo-sectioning, or focused ion beam milling [92]. These methods are adaptable for various sample types and are essential for obtaining precise spectral data while minimizing artifacts introduced by the microscope.

Researchers can optimize experimental performance and obtain meaningful results by tailoring their preparation strategies based on an understanding of how infrared light interacts with samples. This approach ensures dependable data acquisition, leading to successful outcomes in infrared microscopy.

Sample mounting plays a critical role in obtaining accurate and precise imaging results [93]. Scientists have developed several mounting methods, including the KBr pellet technique, reflective methods, and attenuated total reflectance (ATR), each suitable for different sample types and infrared imaging modes. Each method offers distinct advantages and presents various challenges depending on the properties of the sample being imaged and the desired outcomes (as summarized in Table 3).

Table 2. A comparative summary of machine learning and artificial intelligence techniques used in infrared microscopy, detailing their applications, advantages, limitations, and key references.

Method	Application in Infrared Microscopy	Advantages	Limitations	Reference
Independent Component Analysis (ICA)	Blind source separation and decomposition of IR microscopy data	Identifies statistically independent components	Assumes statistical independence; Sensitive to noise	[83]
Supervised Learning	Image classification, segmentation, and identification of features in IR microscopy data	High accuracy; Robustness to noise; Human-interpretability	Requires labeled data; Can overfit if training data is small	[84]
Unsupervised Learning	Clustering and dimensionality reduction of IR microscopy data	No labeled data required; Can uncover hidden patterns	Less interpretable; Sensitive to initial conditions	[85]
Convolutional Neural Networks (CNNs)	Feature extraction and image analysis in IR microscopy data	Automatic feature extraction; High accuracy	Require large amounts of data; Computationally expensive	[86]
Support Vector Machines (SVMs)	Classification of IR microscopy data	Robust to outliers; Can handle high-dimensional data	Requires parameter tuning; Not efficient for large datasets	[87]
Principal Component Analysis (PCA)	Dimensionality Reduction and visualization of IR microscopy data	Reduces data complexity; Retains essential information	Assumes linear relationships; Can be sensitive to noise	[85]
Transfer Learning	Applying pre-trained models to IR microscopy data analysis	Accelerates training time; Reduces data requirements	May not be ideal for highly specific tasks	[84]
Reinforcement Learning	Optimizing sample positioning and data acquisition in IR microscopy experiments	Adapts to new situations; can optimize for multiple objectives	Requires careful reward function design; Can be slow to converge	[88]
Generative Adversarial Networks (GANs)	Synthesizing Realistic Infrared Microscopy Data for training and evaluation	Generates high-quality data; Can improve model performance	Can be difficult to train; Sensitive to model architecture	[89]
Autoencoders	Denoising, compression, and feature extraction of IR microscopy data	Can reduce noise and data complexity	May not retain all relevant information	[90]
Deep Learning	Advanced feature extraction and classification of IR microscopy data	Can model complex relationships; High accuracy	Requires large amounts of data; Can be difficult to interpret	[27]

For deeper insights into the molecular composition or structure of a sample, additional techniques involving chemical or enzymatic modifications, functionalization, or isotopic labeling can be employed.

Table 3. A systematic comparison of sample preparation techniques in infrared microscopy, highlighting their applications, advantages, limitations, and key references. The table aims to guide researchers in selecting methods aligned with their experimental needs and to provide a comprehensive understanding of the diverse techniques.

Technique	Application in Infrared Microscopy	Advantages	Limitations	Reference
Cryo-sectioning	Preserving hydrated samples for IR microscopy	Maintains sample integrity; Reduces thermal degradation	Requires cryocapable equipment; Freezing artifacts	[94]
KBr pellet technique	Creating transparent, homogeneous samples for IR microscopy	Simple preparation; Good for solid and powdered samples	Requires large sample amount; Limited sensitivity	[95-97]
ATR (Attenuated Total Reflectance)	Minimizing sample preparation for IR microscopy	Minimal sample preparation; Works with various sample types	Limited penetration depth; Requires ATR accessory	[98]
Microtoming	Slicing samples into ultra-thin sections for IR microscopy	Enables high-resolution imaging; Reduces sample distortion	Requires specialized equipment; Skilled handling	[99]
Embedding	Stabilizing Samples in a solid matrix for IR microscopy	Preserves sample integrity; Facilitates sectioning	Possible matrix interference: Matrix selection is critical	[100]
Drop-casting	Depositing Liquid Samples on a substrate for IR microscopy	Simple preparation; Suitable for liquid samples	Requires controlled drying; Potential for uneven deposition	[101]
Air-drying/spin-coating	The formation of thin films of liquid samples for IR microscopy	Fast sample preparation; Good for liquid samples	Requires equipment; Can introduce artifacts	[102]
Liquid cells	Analyzing liquid samples in situ using IR microscopy	Enables in situ analysis; Minimizes sample disturbance	Requires specialized equipment; Limited spatial resolution	[103]
Thin sectioning	Preparation of thin, uniform samples for IR microscopy	Enables high spatial resolution; Minimizes scattering	Requires skilled handling; Time-consuming	[104]

Addressing Technical Challenges in Infrared Microscopy for Innovations in Analytical Excellence

Infrared microscopy provides a robust method for analyzing the molecular structure and composition of various materials with enhanced precision [105]. Despite its numerous advantages, researchers face specific challenges that can hinder their progress, as detailed in Table 4. This review aims to clarify the complexities associated with infrared imaging methods and offer recommendations for effectively addressing them to optimize their potential.

One significant challenge is the absorption of infrared radiation by water molecules, which can attenuate signals or obscure spectral features in examined samples [106]. To mitigate this issue, maintaining optimal humidity levels during experiments and adhering to rigorous sample preparation protocols are crucial. Additionally, mathematical algorithms can be applied to correct distortions caused by water absorption [107].

Another limitation is the diffraction limit of infrared microscopy, which researchers have overcome using near-field techniques such as super-resolution imaging technologies [108].

Addressing the challenge of low signal-to-noise ratio (SNR) in infrared microscopy data acquisition involves implementing various strategies. These include optimizing microscope configurations, using advanced detectors and signal processing techniques, and applying noise reduction algorithms alongside spectral averaging [109].

Table 4. A comprehensive examination of primary obstacles in infrared microscopy, presenting practical resolutions alongside their respective benefits and constraints. This study addresses a range of topics, including light scattering and data acquisition time, by systematically evaluating the advantages and disadvantages of various solutions. The analysis is supported by relevant references. The objective is to offer a detailed analysis of the difficulties and potential solutions in the field.

Challenge	Solution(s)	Advantages	Limitations	Reference
Light scattering by the sample	Thin sectioning; ATR (Attenuated Total Reflectance); Focal plane array (FPA) detector	Improved signal-to-noise ratio; Enhanced resolution	Sample preparation; Equipment requirements	[110]
Low spatial resolution due to diffraction limit	Near-field infrared microscopy; super-resolution techniques; Confocal infrared microscopy	Higher spatial resolution; Better imaging capabilities	Complex techniques; Specialized equipment	[111]
Incomplete sample coverage	Hyperspectral imaging; Automated stage movement; Stitching algorithms	Comprehensive sample analysis; Improved data quality	Data processing; Instrumentation requirements	[112]
Challenges in data analysis	Machine learning and AI algorithms for image analysis; Hyperspectral unmixing algorithms; Spectral libraries	Improved data interpretation; Enhanced analysis speed	Algorithm development; Computational requirements	[113, 114]
Variability in sample composition	Multivariate analysis; Principal component analysis (PCA); Partial least squares (PLS) regression	Better understanding of complex samples; Improved quantification	Requires extensive computational analysis	[115]
Weak or overlapping infrared signals	Subtraction techniques; Multivariate curve resolution; Two-dimensional correlation spectroscopy	Improved spectral resolution; Clearer identification of signals	Requires advanced data processing techniques	[116, 117]
Sample fluorescence interference	Time-gated detection; Fluorescence quenching agents	Reduced fluorescence interference; Improved spectral quality	Additional sample preparation; Equipment requirements	[118]
Non-uniform sample thickness	Automated focus adjustment; Depth profiling	Enhanced image quality; Accurate measurements	Additional equipment requirements; Increased analysis time	[119]
Limited sensitivity	Enhanced sensor technologies (InGaAs, graphene-based detectors); Signal amplification methods	Increased sensitivity; Improved detection capability	Equipment costs; Complexity	[120]
Sample damage due to high-energy radiation	Cryo-sectioning; Rapid data acquisition techniques; Minimizing exposure time	Preservation of sample integrity; Reduced damage	Specialized equipment; Sample preparation	[121]
Long data acquisition times	Fourier transform infrared (FTIR) microscopy; Quantum cascade laser (QCL) microscopy; FPA detectors	Faster data acquisition; Higher throughput	Equipment costs; Complexity	[41]

Applications of Infrared Microscopy Across Disciplines

Elucidating Molecular Mechanisms in Biomedical and Life Sciences via Infrared Microscopy

Infrared microscopy has significantly transformed the fields of biomedical and life sciences by offering insights into the molecular complexities of biological systems [122]. This technology advances disease diagnostics and cellular imaging through its label-free [123], non-invasive [124] approach to sample analysis. Its transformative capabilities have a profound impact on patient care, providing precise diagnoses for conditions such as cancer, neurodegenerative disorders, and infectious diseases in a swift and minimally invasive manner.

Real-time visualization of cellular components and exploration of dynamic molecular processes are facilitated by infrared microscopy [125]. In drug discovery and development, this powerful tool aids in target recognition, monitors cellular responses to drugs, evaluates safety profiles, and assesses the efficacy of therapeutic compounds, thereby advancing the development of effective therapies [88].

Infrared microscopy also plays a crucial role in tissue engineering and regenerative medicine [126]. Its nondestructive and label-free imaging capabilities reveal components of cellular and extracellular matrices essential for developing innovative tissue scaffolds [127], optimizing stem cell differentiation protocols [128], and monitoring the integration of engineered tissues within host organisms [129], thereby advancing personalized regenerative therapies.

Similarly, infrared microscopy serves environmental and plant sciences by enabling researchers to explore the molecular composition of environmental samples [110], assess the effects of pollutants on natural systems [130], and understand the molecular mechanisms involved in plant growth [131], development, and responses to stressors.

Infrared microscopy presents numerous possibilities across various fields, as highlighted in Table 5.

Material Science: Investigating the Microstructure of Materials

Infrared microscopy has significantly enhanced the field of material science by providing insights into microstructure, composition, and properties [145]. This versatile tool finds applications in polymer science and the investigation of nanomaterials like nanoparticles and 2D materials, offering valuable information about chemical composition, molecular orientation, and morphological characteristics. As a result, our understanding of the relationship between structure and properties has markedly improved [146], driving the development of innovative materials with customized properties for diverse applications.

Infrared microscopy enables high-resolution imaging and spectroscopic analysis at the nanoscale, facilitating a deeper understanding of size, shape dynamics, and surface properties [46]. It is particularly valuable for analyzing thin films or coatings used in solar cells, sensors, and protective coverings, providing detailed information on parameters such as thickness, chemical composition, and interfacial interactions [147]. This critical data empowers improvements in these elements, leading to enhanced efficiencies across various fields.

This transformative imaging technique has made significant strides in materials science by offering non-destructive evaluation of flaws such as cracks, voids, and inclusions in materials. Additionally, it provides valuable insights into temperature-dependent phenomena like phase transitions and thermal properties [110], enabling the development of materials with specific thermal characteristics [148].

Table 5. A comprehensive review of the use of FTIR, Raman, and Near-field Infrared Microscopy techniques in biomedical research. It details applications across various sample types and research areas, such as neurodegenerative diseases and pathogen identification, discussing the advantages and limitations of each technique within specific contexts, supported by relevant citations. The aim is to provide a comparative perspective on the utility of these microscopy methods in biomedicine.

Application	Technique	Sample Type	Advantages	Limitations	Reference
Neurodegenerative disease characterization	FTIR, Raman	Brain tissue sections	Detection of protein misfolding; Identification of biochemical markers	limited spatial resolution; Sample variability	[132]
Drug delivery and tracking	FTIR, Raman	Cells, tissues	Monitoring drug distribution; Evaluating drug efficacy and safety	Limited penetration depth; Sensitivity to high molecular weight compounds	[133]
Stem cell differentiation	FTIR, Raman	Stem cells	Non-destructive; Label-free; Real-time monitoring; Quality control	Limited sensitivity for rare cell types; Interpretation of complex spectra	[134]
Imaging of lipid distribution	FTIR, Raman	Cells, tissues	High chemical specificity; High spatial resolution	Limited penetration depth; Sensitivity to sample thickness	[135, 136]
Monitoring cell apoptosis	FTIR, Raman	Cells	Early detection of apoptosis; Label-free; High specificity	Limited sensitivity for early-stage apoptosis; Interpretation of complex spectra	[137]
Investigation of protein structure	FTIR, Raman	Protein samples	Determination of secondary structure; Evaluation of folding/unfolding	Limited sensitivity for low-abundance proteins; Challenges in data interpretation	[138]
Analysis of extracellular matrix composition	FTIR, Raman	Tissue sections	Detection of matrix components; Analysis of spatial distribution	Challenges in data interpretation; Limited spatial resolution	[139]
Pathogen identification	FTIR, Raman	Bacteria, fungi, viruses	Rapid identification; Label-free; Minimal sample preparation	Differentiation of closely related species; Limited spectral libraries	[140]
Atherosclerosis assessment	FTIR	Arterial tissue	Evaluation of plaque composition; Identification of vulnerable plaque	Invasive sample collection; Data processing and analysis	[141]
Metabolomics	FTIR, Raman	Cells, tissues, biofluids	Detection of metabolite signatures; Metabolic pathway analysis	Overlapping spectral features; Standardization of data processing	[142]
Cancer diagnosis and grading	FTIR, Raman, Near-field IR microscopy	Tissue sections	Non-destructive; Label-free; Molecular information for diagnosis; High sensitivity and specificity	Sample preparation; Data interpretation; Standardization	[143]
Imaging of subcellular organelles	Near-field IR microscopy	Cells	High spatial resolution; Label-free; Nanoscale imaging	Complex techniques; Limited penetration depth	[144]

Environmental Monitoring: Analyzing Microplastics and Atmospheric Particles

Infrared microscopy is revolutionizing environmental monitoring by providing valuable insights into the composition, distribution, and potential impacts of microplastics and atmospheric particles [148]. This section explores the diverse applications of infrared microscopy in enhancing our understanding of environmental concerns and facilitating efficient remediation strategies.

Microplastics, known for their environmental harm, are increasingly detectable through this advanced technique. Infrared microscopy offers crucial information about the size, shape, and chemical composition of particles. This data is essential for assessing associated risks and developing effective pollution reduction strategies [149].

In the realm of atmospheric particles, infrared microscopy plays a vital role. Researchers can glean valuable insights into their chemical structure, morphology, and size distributions. This information is critical for devising strategies to manage air quality and mitigate climate change impacts.

In bioaccumulation studies, infrared microscopy facilitates the investigation of microplastic accumulation and ecotoxicological implications [150]. Such insights are indispensable for safeguarding ecosystems from further damage.

Moreover, infrared microscopy proves highly efficient in detecting trace levels of contaminants such as persistent organic pollutants and heavy metals [151]. As such, it has become the preferred method for monitoring environmental conditions with its unique sensitivity and specificity, contributing significantly to environmental protection efforts.

Advanced Infrared Microscopy Techniques in the Analysis and Preservation of Art and Cultural Heritage

Infrared microscopy has significantly enhanced the examination of art and cultural heritage, providing scholars and conservators with a powerful tool to uncover intricate details of artworks and illuminate their artistic evolution over time [152]. This section delves into the various applications of infrared microscopy in art conservation, emphasizing its role in offering valuable insights into masterpieces and supporting their long-term preservation.

Reflectography stands out as a highly valuable non-invasive imaging technique used by experts to reveal hidden layers beneath the surface of artworks [153]. It enables the detection of underdrawings, pentimenti, and other subtle details that offer profound insights into an artist's creative process. These revelations serve as invaluable resources for art historians, aiding in authentication and enriching understanding of artistic intentions.

Furthermore, infrared microscopy enables the analysis of the chemical composition and distribution of pigments used in historical artworks [154]. Its capability to detect early signs of deterioration, such as cracks, delamination, and discoloration, is crucial for proactive conservation efforts [154, 155]. This technology plays a pivotal role in preserving cultural heritage by facilitating informed conservation decisions and ensuring the longevity of invaluable artworks.

Future Prospects and Ethical Considerations in Infrared Microscopy

Emerging Trends and Future Research Directions in Infrared Microscopy

The development of smaller and more portable designs has the potential to enhance the availability of advanced imaging capabilities for researchers in various contexts, including remote or resource-limited areas. This expansion could significantly broaden the application of such technology. However, there are concerns regarding the equitable access to these technologies. Additionally, integrating infrared microscopy with other advanced imaging techniques, such as Raman spectroscopy and X-ray fluorescence, enables comprehensive sample analysis, leading to valuable interdisciplinary advancements. Nevertheless, careful consideration is necessary for data management [156], interpretation, and distribution among

interdisciplinary teams [157].

The integration of artificial intelligence (AI) and machine learning algorithms in infrared microscopy has the potential to revolutionize image analysis [27]. These technologies can accelerate discoveries and foster innovation by facilitating rapid and accurate identification of patterns and features within complex datasets. The rapid advancement of AI technology necessitates assessment of data privacy regulations, transparency standards, and potential biases in AI-driven analyses [158]. As infrared microscopy progresses swiftly, researchers and practitioners must address the ethical implications associated with its use, including obtaining informed consent and preventing misuse of information.

Achieving optimal utilization of the benefits offered by this powerful imaging technique, while effectively managing potential risks, requires collaborative efforts among all stakeholders, including the incorporation of AI-driven analyses [159].

Balancing Innovation and Privacy: Ethical Implications

Researchers must implement strict protocols for handling sensitive information, particularly when dealing with human tissue samples or proprietary substances. This is crucial to protect the rights and autonomy of individuals who consent to participate in research studies. Transparency is essential to establish trust and ethical standards. Providing study participants with a clear explanation of how infrared microscopy will be used enables them to make informed decisions about their participation in the investigation. Adhering to these guidelines prevents personal security breaches while engaging in significant scientific exploration using infrared microscopy.

Infrared microscopy has significantly advanced our understanding of the environment, providing unprecedented knowledge regarding the structure and dynamics of molecules. However, the vast potential of this technology also requires a higher level of accountability in safeguarding the privacy and security of individuals' data. To ensure ethical and responsible utilization of these advancements, researchers must establish precise research guidelines for infrared microscopy. Through open dialogue, we can collectively address the ethical implications of advances in infrared microscopy. This collaborative effort aims to promote responsible practices and optimize the potential for innovative discoveries. It facilitates a comprehensive analysis of the optimal balance between technological advancement and ethical concerns, such as genetic discrimination and unauthorized access to confidential data.

CONCLUSION

Infrared microscopy has emerged as a versatile and powerful technique with transformative potential across various scientific disciplines. This review provides a comprehensive examination of the methodologies, challenges, and emerging trends in infrared microscopy, offering a detailed analysis of the current landscape in this field. We have discussed key aspects such as sample preparation techniques and computational strategies for data interpretation, illustrating how recent advances are addressing existing challenges and enhancing the effectiveness and efficiency of this technology.

Furthermore, we have explored the diverse applications of infrared microscopy, highlighting its significant contributions to biomedical research, environmental monitoring, material science, and cultural heritage conservation. These advancements underscore its pivotal role in advancing scientific knowledge and facilitating innovative solutions in multiple domains.

As infrared microscopy continues to evolve with technological progress, ethical considerations surrounding data privacy and equitable access have become increasingly important. It is crucial for researchers to maintain ethical rigor and methodological robustness, particularly as the field integrates advancements like artificial intelligence. By leveraging infrared microscopy to its fullest potential, we can foster both scientific advancement and ethical responsibility.

This review serves as a testament to the achievements in the field of infrared microscopy and aims to guide future research efforts. It emphasizes the importance of a balanced approach that prioritizes both technological innovation and ethical considerations. Through collaborative efforts, the near future holds promise for groundbreaking discoveries and practical applications in infrared microscopy, shaping a remarkable era of scientific progress.

Conflicts of interest

The authors have stated that there is no conflict of interest associated with the publication.

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التحليل الطيفي المجهري بالأشعة تحت الحمراء: دراسة متعددة المجالات للتقنيات، الاستخدامات التطبيقية، والقضايا الأخلاقية

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ملخص

المجهرية الطيفية باستخدام الأشعة تحت الحمراء تعتبر واحدة من التقنيات الصاعدة التي لها تطبيقات واسعة في مجموعة متنوعة من الميادين العلمية. تستهدف هذه الدراسة متعددة التخصصات فحص متكامل للأساليب، التحديات، والابتكارات في هذا المجال. نقدم نظرة على أهمية طرق إعداد العينات، بما في ذلك التجزئة الكريوجينية والانعكاس الكلي المُضعف، في تحسين جودة البيانات. توجه الدراسة الأضواء على التحديات التي تواجهها عمليات الحصول على البيانات وتفسيرها، مع التركيز على حلول متقدمة مثل الخوارزميات المبنية على التعلم الآلي والمستشعرات الحساسة. الدراسة تكشف عن أوسع استخدامات المجهرية الطيفية في مجالات متعددة مثل الأبحاث الطبية، ومراقبة البيئة، وعلوم المواد، وحفظ الفنون. وتتعدى الدراسة الجوانب التقنية لتناقش التوجهات الجديدة، مثل دمج التكنولوجيا مع الذكاء الصناعي، والقضايا الأخلاقية كحماية البيانات والموافقة المُسبقة والمُستنيرة. تُقدم الدراسة مرجعاً شاملًا للباحثين، سواء كانوا متمرسين أو مبتدئين، عبر تقديم رؤية شاملة تجمع بين التطورات الأسلوبية والمعايير الأخلاقية.

الكلمات الدالة: التقنيات المجهرية الطيفية بالأشعة تحت الحمراء، إعداد العينات، تطبيقات متعددة المجالات، القضايا الأخلاقية، الخوارزميات المبنية على التعلم الآلي والذكاء الصناعي.

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تاريخ استلام البحث 2023/9/3 وتاريخ قبوله للنشر 2024/1/16.

Chitosan/ Alginate/ Gelucire in-situ Gelling System for Oral Sustained Delivery of Paracetamol for Dysphagic Patients

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ABSTRACT

The study aims to formulate an oral in-situ gel for sustained paracetamol delivery, targeting pediatric and geriatric patients. A blend of sodium alginate, chitosan, and Gelucires was used to create the gel. Characterization techniques, such as rheology and in vivo bioavailability tests on rat models, were employed. The in-situ gel transitioned into a gel-matrix system in 0.1N HCl, effectively controlling the release of paracetamol at different pH levels (1.2, 5.4, and 6.8). Gels made solely of sodium alginate or sodium alginate-chitosan exhibited rapid drug release at pH 6.8. The formulation containing paracetamol in a Gelucire (G33/01):3-3% sodium alginate - chitosan ratio of 1:1:4 w/w showed an extended drug release time of over 8 hours. Bioavailability in rats revealed a higher time to maximum concentration (Tmax) and lower peak concentration (Cmax) but comparable mean residence time (MRT) and area under the curve (AUC0-∞) to commercial formulations. The gel's synergistic blend of chitosan, sodium alginate, and Gelucire G33/01 ensures a sustained release of paracetamol, making it a promising drug delivery system for vulnerable populations like children and the elderly.

Keywords: *In situ* gel matrix system; chitosan; alginate; Gelucires; Paracetamol; Sustained release.

INTRODUCTION

In-situ gelling systems are utilized for targeted drug delivery and employ different mechanisms of gelation in vivo. These mechanisms are designed to meet specific requirements for drug release and anatomical targets [1-8]. These systems transform from a liquid sol-state to a gel upon encountering specific environmental triggers, such as temperature, enzymes, pH, or ions [9-13]. Temperature-induced gelation involves polymers that undergo a transition from a liquid state to a gel state at body temperature [14], making them suitable for various applications where a minimally invasive approach is preferred. pH-induced gelation refers to the process where polymers undergo sol-to-gel transitions in response to pH variations [15], allowing for precise drug release in regions characterized by distinct pH levels. Enzyme-triggered gelation is a targeted approach that responds to specific enzymes found in particular tissues [16], enabling precise drug delivery in localized areas. Ion-induced gelation refers to the process in which polymers undergo gelation when exposed to specific ions [17]. This phenomenon has practical applications in various fields, such as wound healing, where the concentration of ions at the site of application fluctuates. Each mechanism provides distinct benefits for precise and controlled drug administration, influenced by the environmental factors of the specific tissue and the interplay between the drug and

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polymer. Nonetheless, the intrinsic mechanical fragility of the polymers involved often results in premature erosion, prompting research into methods like polymer blending, grafting, cross-linking, and mixed-matrix approaches to enhance their structural integrity. Blending polymers is an especially effective yet economical strategy to modulate the physical properties of in-situ gels, making them highly relevant for oral administration in populations with dysphagia and those of pediatric or geriatric age. Paracetamol (PA), commonly delivered through tablets or liquids, offers an excellent model drug for these systems, as its extended release has been successfully achieved via in-situ gel formation using polymers of both natural and synthetic origins [18-23]. This study introduces a novel blend consisting of Gelucire® (G), a highly hydrophobic lipid material, with a dual hydrophilic polymer matrix of chitosan (CS) [24, 25] and sodium alginate (SA) [26]. This blend is designed to form an in-situ gel matrix in the stomach for sustained PA release, addressing the limitations associated with the rapid diffusion of hydrophilic drugs [27]. Both CS and SA are biocompatible polysaccharides with unique ionic properties [28-37], while Gelucire® serves as an amphiphilic lipid material with specific fat-soluble or water-dispersible characteristics [38].

MATERIALS AND METHODS

Sodium alginate was obtained as a gift from AL Taqaddom Pharmaceutical Industries Co. (Protanal® LF 120M, Guluronic acid: 35-45% and Mannuronic acid: 55-65%, Lot No.: S20653, FMC BioPolymer A/S, Drammen, Norway). Chitosan was obtained as a gift from the Jordanian Pharmaceutical Manufacturing Co. LTD., JPM (250 KDa with 96% degree of deacetylation, JBICHEM, Shanghai, China). Chitin was obtained as a gift from JPM (200 *M*m, JBICHEM, Shanghai, China). Gelucire® (a saturated polyglycolysed glycerides; Gelucire® grades 33/01, 39/01, and 43/01 were a gift from Gattefosse, France). Paracetamol (Standard of PA, Water Content: 0.1%, Potency: 99.2%) was kindly supplied from Hikma, Jordan. Revanin® (250 mg/ 5 ml) is a commercially available paracetamol suspension manufactured by the Arab Pharmaceutical Manufacturing Co. LTD., APM, Salt, Jordan. All other chemicals and reagents used were either of analytical or pharmaceutical grades.

Preparation of Paracetamol Gel Formulations

Paracetamol: Alginate and Paracetamol: Alginate-Chitosan Formulations:

Three formulations with SA alone were prepared at concentrations of 3%, 4.5%, and 6% (D1, D2, D3) as shown in Table 1.

Table 1 The composition of the in-situ gel formulations (D1, D2, D3, N1, N2, and N3) containing Paracetamol (PA), Sodium Alginate (SA), and Chitosan (CS).

Formula	Contents Ratio w/w	PA (g)	SA colloidal dispersion			The Polymeric Phase		
			3 % w/w (g)	4.5 % w/w (g)	6 % w/w (g)	SA-CS 3-3 % w/w (g)	SA-CS 4.5-4.5% w/w (g)	SA-CS 6-6% w/w (g)
D1	1:4	10	40	-	-	-	-	-
D2	1:4	10	-	40	-	-	-	-
D3	1:4	10	-	-	40	-	-	-
N1	1:4	10	-	-	-	40	-	-
N2	1:4	10	-	-	-	-	40	-
N3	1:4	10	-	-	-	-	-	40

Binary combinations of sodium alginate with chitosan (SA-CS) were prepared at concentrations of 3-3%, 4.5-4.5%, and 6-6% (N1, N2, N3). These combinations were created by dissolving the respective polymers in de-

ionized water, allowing overnight stabilization at 4°C, and subsequently adding paracetamol. The mixtures were then homogenized under specific conditions.

Paracetamol: Gelucire: Chitosan-Alginate Formulations:

Formulations F01-F18 were generated using various Gelucires and SA-CS combinations, as shown in Table 2.

Table 2 The composition of gel formulations (F01 – F18) containing Paracetamol (PA), Gelucire (G), Sodium Alginate (SA), and Chitosan (CS).

Grade of Gelucire®	PA : G : SA-CH Gel					
	(1 : 1 : 4) w/w Ratio			(1 : 1 : 8) w/w Ratio		
	(SA-CS) % w/w			(SA-CS)) % w/w		
	(3-3%)	(4.5-4.5%)	(6-6%)	(3-3%)	(4.5-4.5%)	(6-6%)
G 43/01	F1	F2	F3	F4	F5	F6
G 39/01	F7	F8	F9	F10	F11	F12
G 33/01	F13	F14	F15	F16	F17	F18

A lipidic phase was initially prepared by melting Gelucire and incorporating PA. This phase was combined with different polymeric phases (3-3%, 4.5-4.5%, or 6-6% w/w CS-SA) using a mechanical stirrer (RZR 2041, Heidolph, Germany) at 300 rpm.

Paracetamol: Gelucire: Alginate Formulations:

Three formulations, namely F19, F20, and F21, were prepared without Chitosan, utilizing a 1:1 w/w lipidic phase of PA: G43/01, PA: G39/01, or PA: G33/01 and a 3% SA colloidal dispersion. These were heated and allowed to stabilize overnight at 4°C.

Paracetamol: Gelucire: Chitin-Alginate Formulations:

Formulations F22, F23, and F24 were prepared by replacing chitosan with chitin in a 3-3% w/w ratio, precisely replicating formulations F1, F7, and F13. Each formulation was prepared in triplicate to ensure consistency and reliability.

Rheological Studies

Viscosity measurements were performed at 25 °C with a cone and plate viscometer (Anton Paar Physica MCR 301, Graz-Austria, Type P-PTD 200-62). The viscometer had a cone of 0.994˚ angle and 50.005 mm diameter. Each gel formulation was tested in triplicate over a shear rate range of approximately 2-100 s^{-1} with a gap width of 0.05 mm. Tested each gel in triplicates.

Measurement of *in Vitro* Drug Release

The release of PA from the gel formulations was conducted using the USP paddle method (apparatus II). An appropriate amount of each formulation, equivalent to 250 mg of PA, was accurately weighed and introduced into the dissolution medium using a tip-free disposable syringe. The dissolution medium was maintained at 37±0.5°C with an agitation rate of 50 rpm. To mimic the physiology of the gastrointestinal tract, a gradient pH-dissolution media of 1000 ml volume was employed, starting with 0.1N HCl for 2 hours, changing to pH 5.4 for an additional 2 hours by adding 16.9g trisodium phosphate salt, and finally changing to pH 6.8 by adding 5.4g of the same salt until the end of the test. At predetermined time intervals, 5 ml samples were withdrawn and immediately replenished with pre-warmed fresh medium. Subsequently, the PA content in the samples was analyzed spectrophotometrically at λmax 243 nm. The results, obtained from six determinations, were expressed as the percentage of PA released (USP 30-NF 25, 2007). Each test was performed in triplicate to ensure accuracy and reproducibility.

Elucidation of the Release Mechanism

The drug release pattern was evaluated using four model-dependent kinetic models: zero-order release kinetics, Higuchi's square root of time equation [39], Korsmeyer–Peppas power law equation, and Hixson–Crowell's cube root of time equation [40, 41]. The correlation coefficient, R^2, values evaluated the goodness of fit. The complete dataset of dissolution time was utilized to analyze and elucidate the release mechanism.

Animal Experiments

Twelve male Sprague Dawley rats, weighing 250–315 g, were procured from Al-Yarmouk University Biological Center (Irbid, Jordan) and housed in the Petra University Animal Care Unit (Amman, Jordan) under standard conditions of temperature (22±2°C) and humidity. The rats were allowed to acclimate for a period of 10 days before commencing the experiments. All animal procedures adhered to the guidelines of FELASA (Federation of European Laboratory Animal Science Associations), and the study protocol was reviewed and approved by the Animal Care Committee of the Scientific Research Council of Petra University (approval number: 1223).

On the day of the experiment, the rats underwent a 24-hour fasting period with free access to water. The rats were randomly divided into two groups, with six rats in each group. The first group was administered a 30 mg/kg oral dose of the PA-gel preparation via a metal oral gavage needle (Harvard Apparatus, UK). The second group received a similar dose of a PA commercial suspension, Revanin® (7.5 mg in 0.6 ml, equivalent to 30 mg/kg).

At predetermined time intervals, namely 0, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 hours, blood samples were collected for each group by pooling one blood drop obtained from the tip of the tail (around 35 µl blood/drop) from each rat. The pooled samples were collected in 0.5 ml EDTA blood tubes and centrifuged at 3000 rpm for 5 minutes. The plasma was then collected and stored at -20°C until analysis. The experiment was repeated (n=6) to ensure the reliability and consistency of the results.

In situ Gelling Ability Examination

The animals underwent a 24-hour fasting period with free access to drinking water prior to the administration of the experimental formula using a metallic oral gavage needle. The gel-forming capacity of the formula was assessed by administering approximately 1.3 g and 0.33 g of the formula into the stomachs of rats and mice (n=3 for each group).

Paracetamol Assay

The plasma samples were assayed using HPLC (Shimadzu LC-10A with a Shimadzu SPD-10A detector at a wavelength of 254 nm) according to a previously described method with minor modifications [42]. On the day of analysis, the samples were vortexed for 1 minute, from which 0.150 ml was withdrawn, and the volume was completed with water to 0.5 ml. 50 µl of a 30% perchloric acid solution was added to each tube to precipitate proteins. The samples were vortexed for 1 minute and centrifuged at 6000 rpm for 10 minutes. Finally, 20 µl of the supernatant was injected into a column (150 mm × 4.6 mm i.d. and packed with Inertsil-ODS). Elution was carried out using the KH_2PO_4 (0.1M)-Isopropanol-tetrahydrofuran system in a ratio of 100:1.5:0.1 v/v, and the pH was adjusted to 3.7 using phosphoric acid.

Pharmacokinetic Analysis

The values of the maximum PA plasma concentration (Cmax) and the time of its occurrence (Tmax) were obtained directly from a concentration–time profile. For other pharmacokinetic parameters, the concentration-time data were analyzed using computer-based pharmacokinetic software, WinNonlin® version 5.2.1 (Pharsight Corporation, Mountain View, CA, USA). The area under the plasma level–time curves (AUC) and moment plasma level–time curves (AUMC) were calculated by the trapezoidal method, and the ratio of AUC and AUMC was used to estimate the mean residence time (MRT) of the drug. The program used a minimum of three data points to compute the terminal elimination rate constant (Kel).

Statistical Analysis

Unpaired t-tests, using Prism GraphPad, were carried out to compare the pharmacokinetic parameters of the gel formulation (test) and the commercial suspension (reference). P values of <0.05 were considered significant.

RESULTS AND DISCUSSION

In Vitro Release Studies

In vitro release studies elucidate the efficacy and limitations of various paracetamol (PA) gel formulations. Formulations D1, D2, and D3, which solely incorporated sodium alginate (SA), manifested soft alginic acid gels upon interaction with 0.1 N HCl. However, these SA-only systems showed suboptimal sustained drug release properties, particularly at higher pH values. Figure 1 illustrates this rapid drug release and highlights the suboptimal nature of SA as a sole vehicle for drug delivery.

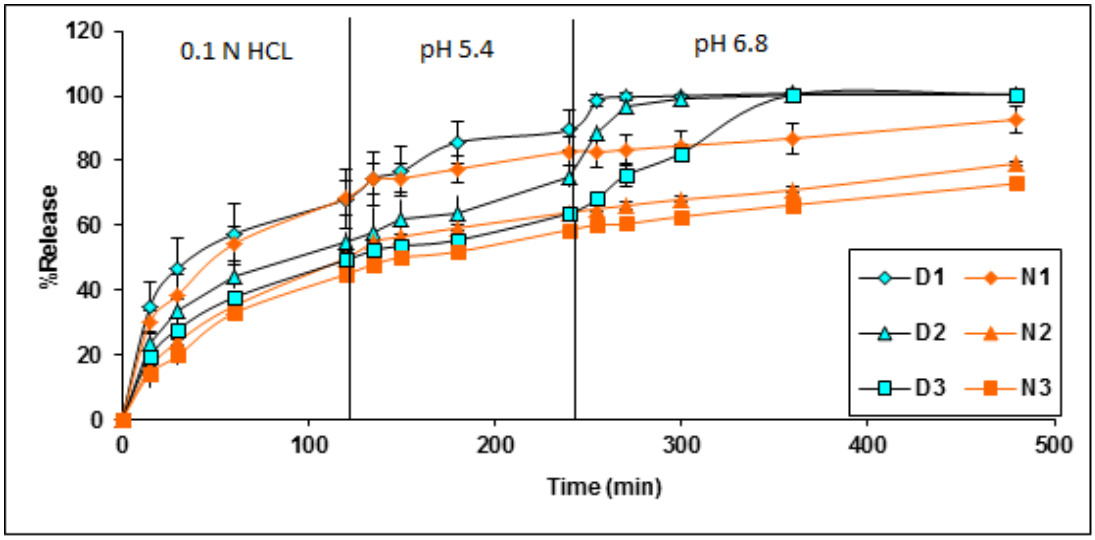

Figure 1 Drug release profiles of PA formulations D1, D2, and D3 with SA as the sole matrix. The data show suboptimal sustained release at higher pH levels.

Such behavior is attributable to the pH-sensitive disintegration of the SA gel in the basic intestinal phase, posing a risk of dose dumping, especially in cases of rapid gastric emptying [43]. To overcome these limitations, SA was blended with chitosan (CS) in formulations N1, N2, and N3. This binary polymeric system displayed advantageous features from both polymers and mitigated their individual drawbacks, thus offering a more effective drug release transition from gastric to intestinal phases [44]. The hybrid SA-CS gel showcased pH-responsive behavior, where the polycationic nature of CS allowed for in-situ gelation in the stomach, prolonging gel structural integrity upon reaching the intestine [45]. Figure 2 shows that among these hybrid formulations, N3 exhibited the most sustained drug release pattern, specifically, a reduction of drug release from 68.48 ± 5.47% to 44.84 ± 1.39% at the end of the acidic stage (2h).

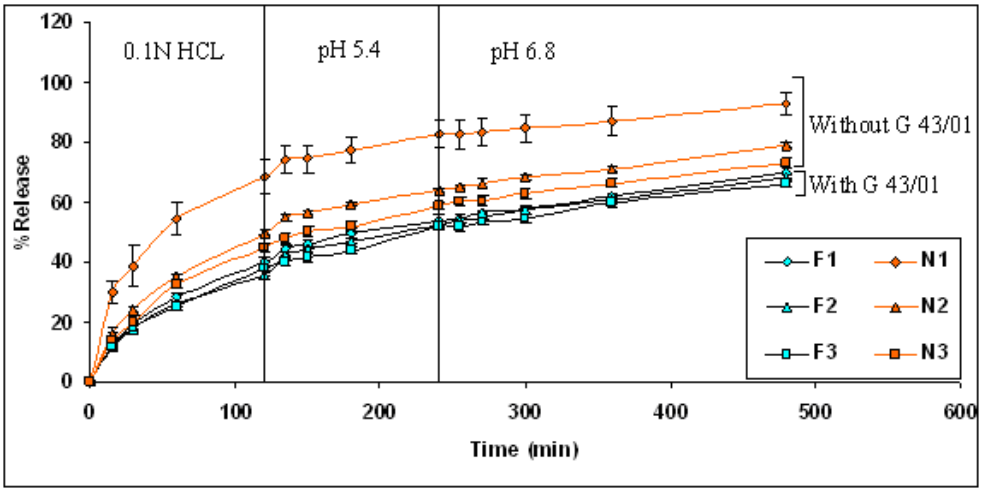

Figure 2 Comparative dissolution profile of in-situ gel formulations containing Gelucire G 43/01 (F1, F2, and F3) with those without Gelucire (N1, N2, and N3).

A novel approach was the inclusion of lipidic material G (G33/01, G39/01, or G43/01) to SA-CS gels in formulations F1-F18. This further reduced drug release, ascribed to changes in matrix hydrophobicity and increased diffusion path length. Notably, formulations incorporating PA:G at a 1:1:4 molar ratio (F1, F2, F3, F7, F8, F9, F13, F14, and F15) demonstrated statistically indistinguishable drug release kinetics, as delineated in Figures 3A, 3B, and 3C.

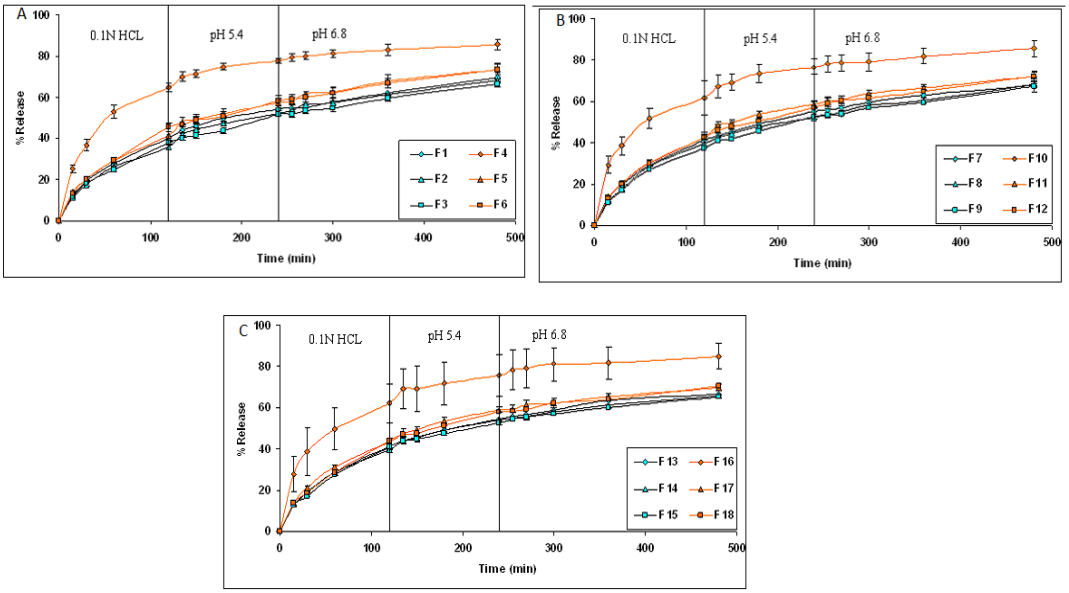

Figure 3 Comparative dissolution profiles of different in-situ gel formulations.

Alterations in SA-CS polymeric concentration from an initial 3%-3% to a subsequent 6%-6% did not induce significant changes in these release parameters. In contrast, formulations with an enhanced 1:1:8 SA-CS ratio (F4, F10, F16) exhibited augmented drug release kinetics, as corroborated by Figures 3A, 3B, and 3C. This increased release is ostensibly attributable to the augmented hydrophilic nature of the polymer matrix. The gel matrices were promptly formed in vitro upon contact with acidic dissolution media (pH 1.2) and also in the in-situ stomachs of rats and mice, as illustrated in Figure 4.

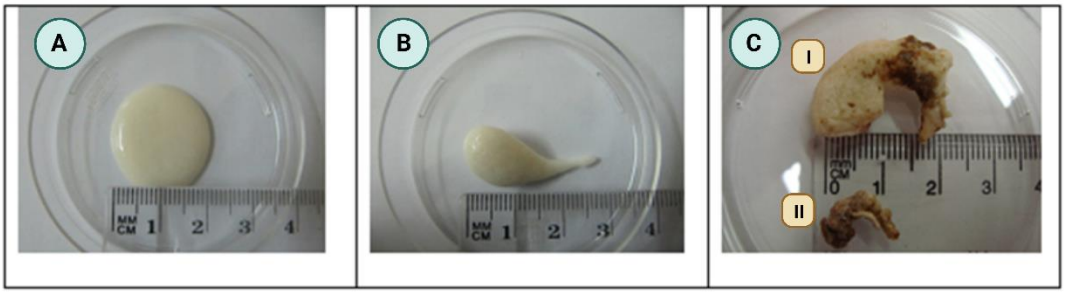

Figure 4 Photographs of the PA: G33/01: CH – AL (1: 1: 4 ratio) gel: (A) before and (B) after addition to the acid media and (C) in-situ rat (I) and mouse (II) stomach 30 min after administration

In vivo studies performed on rats confirmed the rapid in vitro gel formation at acidic pH (pH 1.2). Formulations F1, F7, and F13, which displayed optimal sustained release, were further benchmarked against a commercial PA product (Revanin®, APM), which exhibited complete immediate drug release within 5 minutes, as shown in Figure 5.

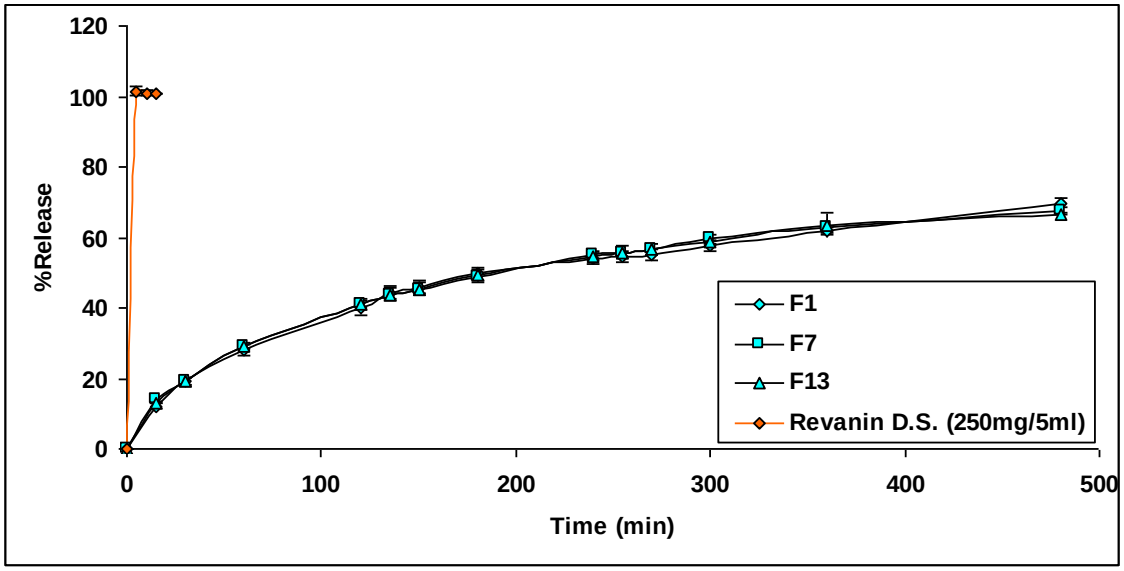

Figure 5 Paracetamol release profiles from Formulas F01, F07, F13 and the commercial PA immediate release suspension (Revanin®, APM)

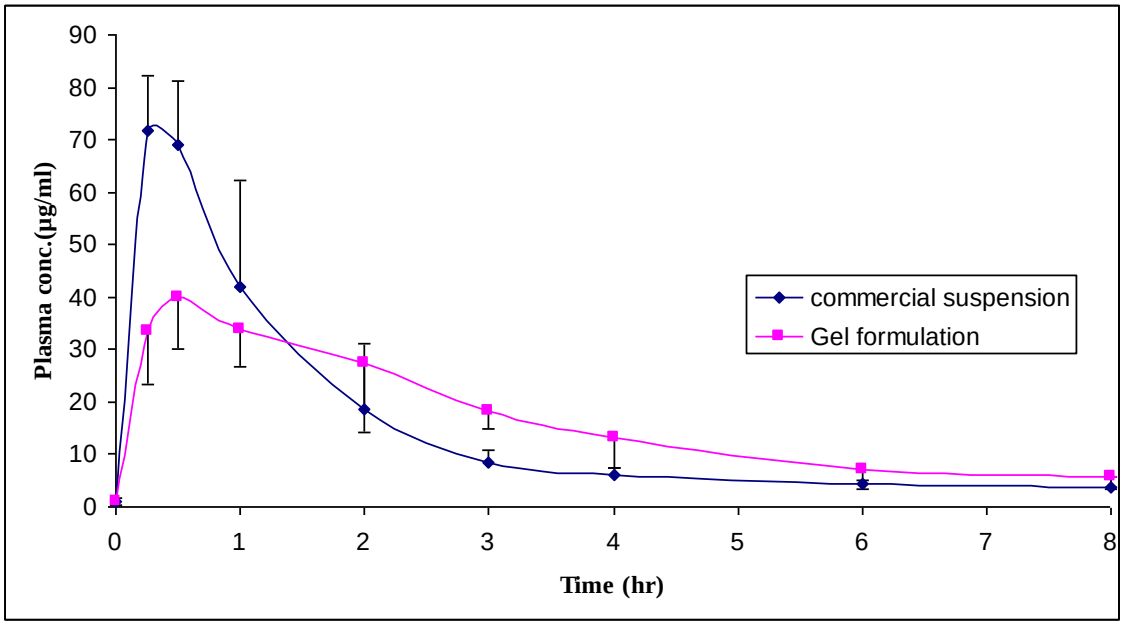

Figure 6 Plasma concentrations of paracetamol in rats after oral administration of commercial suspension and gel formulation (F16). Each value represents the mean ± S.E. of six independent experiments (n=6).

Analysis of the Release Pattern

Table 3 presents the regression parameters obtained after fitting various release kinetic models to the in vitro dissolution data of formulations F1, F4, F7, F10, F13, and F16.

Table 3 Statistical parameters of the various in-situ gel formulations after fitting drug release data into various release kinetic models

Model		F01	F07	F13	F04	F10	F16
Zero-order	r^2	0.8762	0.8728	0.8585	0.7185	0.7729	0.7603
	K_0	0.0012	0.0011	0.0011	0.0012	0.0011	0.0011
First-order	r^2	0.9581	0.9506	0.9376	0.8804	0.9168	0.8999
	K_1	-0.0022	-0.0021	-0.0021	-0.0035	-0.0034	-0.0034
Higuchi	r^2	0.9771	0.978	0.9713	0.8849	0.9217	0.9138
	K_H	0.0315	0.0311	0.0310	0.0333	0.0316	0.0322
Peppas equation	r^2	0.9854	0.9938	0.991	0.9822	0.9897	0.9913
	N	0.5077	0.4838	0.4998	0.4595	0.3647	0.3861
	K_P	-1.4731	-1.4119	-1.4496	-1.1225	-0.9551	-0.9987
Hixson-Crowell	r^2	0.9349	0.9276	0.9139	0.8292	0.8728	0.8571
	K_C	-0.0006	-0.0006	-0.0006	-0.0008	-0.0008	-0.0008

The goodness of fit using different models was ranked in the following order: Korsmeyer-Peppas > Higuchi > first order > Hixson-Crowell cube root law > Zero order. By employing the Korsmeyer-Peppas model, the obtained "n" values for all tested formulations ranged from 0.45 to 0.50. These "n" values indicate that both drug diffusion and polymer relaxation (swelling/erosion) mechanisms are involved in the drug release process.

Viscosity Study

The rheological profiles of the formulated gels have significant implications for their oral administration efficacy. Figure 7 elucidates the shear-dependent viscosity characteristics, revealing a direct correlation between increased viscosity and the melting points of incorporated G constituents.

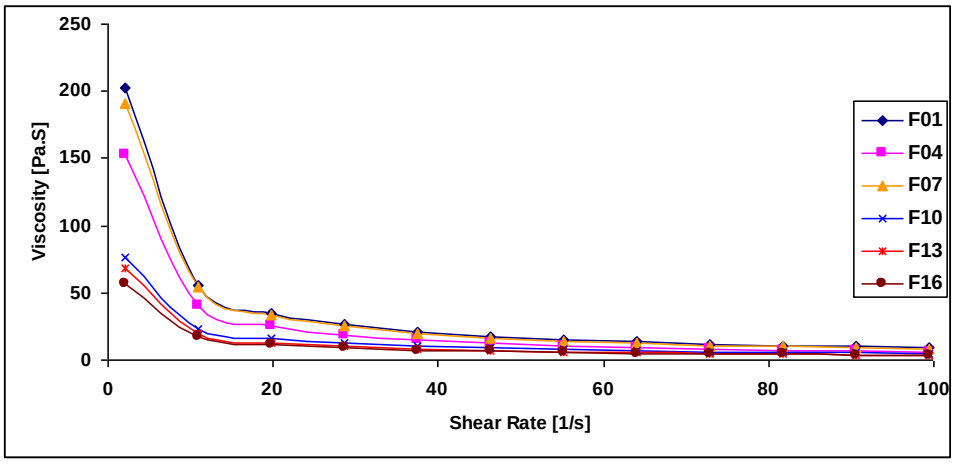

Figure 7 The effect of Shear rate [1/s] dependency of the viscosities of Formulas F01, F04, F06, F10, F13 and F16.

Figure 8 further corroborates the pseudoplastic behavior of the formulations, as evidenced by decreasing apparent viscosities upon incremental shear rate alterations.

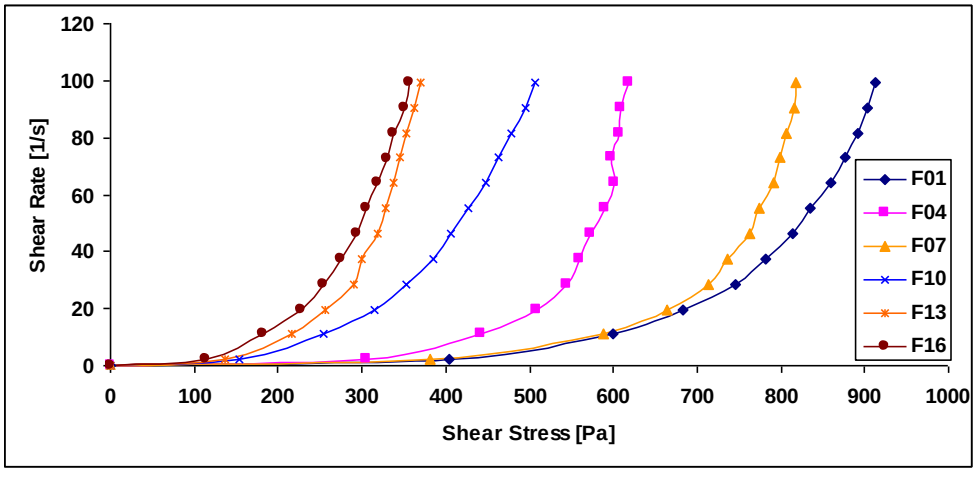

Figure 8 The Flow Curve of Formulas F01, F04, F07, F10, F13 and F16.

Quantitatively, the yield stress values for formulations F16, F13, F10, F4, F7, and F1 ranged from 265 to 750 Pascal-seconds. A pivotal observation was that a two-fold augmentation in the SA-CS polymer ratio resulted in a commensurate decrease in viscosity. In this context, formulations F10, F13, and F16 emerged as favorable candidates for oral administration, attributed to their lower viscosity profiles. Crucially, formulation F16 was subjected to subsequent in vivo evaluations.

***In Vivo* Study**

The release of PA from formulation F16 following oral administration was monitored by determining plasma drug levels. The gelation of this formulation was confirmed by visual observation of the stomach contents, which showed the presence of a distinct matrix mass (as seen in Fig. 4). Figure 6 compares PA levels from the gel with those following oral administration of the commercial suspension. The formed gel was capable of releasing the drug in a sustained manner, providing a relatively consistent plasma concentration profile. The obtained AUC0–∞, MRT, Cmax, Tmax, and t1/2 are summarized in Table 4 along with those published using the same drug and animal models.

Table 4 Comparison of bioavailability parameters of paracetamol administered from the commercial suspension, gel formed in situ in rat stomach (F16)

dosage form	C_{max}	*Tmax*	AUC	MRT	$t_{1/2}$
Gel formulation (F16)	41.0 ± 0.5	0.50 ± 0.08	177.71± 26.4	5. 4 ± 0.7	4.5 ± 1.2
Commercial suspension	74.8 ± 1.2	0.25 ± 0.09	164.3 ± 44.6	4.8 ± 0.9	4.4 ± 0.9

Significantly higher peak time, t_{max} (0.50 ± 0.09 *vs.* 0.25 ± 0.08, *P<0.003*), and lower values of peak concentration, C_{max} (41.0±0.50 *vs* 74.0±1.2, *P<0.001*) were exhibited by the test gel versus the commercial suspension. The area under the curve up to infinite time, $AUC_{0-\infty}$ (177.1±26.4 *vs* 164.3±44.5, *P<0.05*) for the gel and suspension were not significantly different, indicating the similar extent of absorption of the sustained test gel to the reference commercial suspension. The obtained values compare well with those reported earlier (refer to Table 4) in the same animal model.

It is interesting to note the similarity in mean residence times (MRT) between the gel and the commercial suspension. The sustained release effect of the gel formulation results from the gel structure's resistance to the drug's diffusion, whereas that of the suspension arises from the reservoir effect of the suspended particles as they slowly dissolve in the intestine. This result aligns with previous findings [3].

CONCLUSIONS

One of the most commonly used methods to achieve an in-situ gel in the stomach involves combining an anionic polysaccharide with a source of cations, typically calcium. However, excessive calcium intake can be harmful to hypercalcemic patients, and calcium ions may interact unfavorably with certain drugs. This study presents an alternative approach by using a combination of CS and SA without calcium. There is a potential synergistic effect between these two polysaccharides that enhances gel consistency. The mechanism of in-situ gelation relies on the formation of a crosslinked network between the polymers. The addition of hydrophobic Gelucires further enhances gel consistency. These formulations form a gel matrix system immediately upon contact with 0.1N HCl and in the rat stomach in situ. Such a matrix can sustain the release of PA throughout the gastrointestinal tract (65% release after 8 hours). Gelucire® types with similar HLB values and melting points ranging from 33 to 43°C showed no significant differences ($p < 0.05$), likely due to their

similar hydrophobicity. This preparation offers a system with suitable viscosity, easy swellability, prolonged drug action time, and reduced drug administration frequency.

Statements and Declarations

Conflicts of interest: There are no conflicts to declare.

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Authors' contributions

Al-Sayed Sallam: Conceptualization and Formal analysis. Inam Al-Naji, Ruaa Al-Ajeeli: Methodology and Formal analysis. Nidal A. Qinna: Methodology and Resources. Faisal Al-Akayleh, Mayyas Al-remawi, Mai Khanfar: Formal analysis and Writing - Original Draft. Ahmed S.A. Ali Agha: Validation, Software, and Visualization.

Conflict of Interest: The authors declare no conflict of interest.

Statement of Human and Animal Rights

The animal studies were performed after receiving approval of the Institutional Animal Care and Use Committee (IACUC) in the University of Petra (IACUC approval No. A1/9/2021).

Availability of data and materials: Available upon request

Competing interests: The authors declare that none of them have a competing interest.

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نظام التجلئة الموقعي للكيتوزان/الجيلاتين/جيلوسير للتوصيل المُستدام عن طريق الفم للباراسيتامول للمرضى الذين يعانون من صعوبة في البلع

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ملخص

تهدف الدراسة إلى تطوير نظام تركيبة تعطى عن طريق الفم وتكون جل داخل المعدة نتيجة تعرضها لدرجات الحموضة العالية في المعدة ويُحقق توصيل مُستدام للباراسيتامول، مُستهدفًا الفئات العمرية الصغيرة والكبيرة. تم استعمال مزيج من الجينات الصوديوم، الكيتوزان، وجيلوسير. تم التوصيف التركيبة باستخدام تقنيات مثل التفحص الحراري التفاضلي، وطيف الأشعة تحت الحمراء ، واللزوجة. أظهر النظام قدرة على التحكم الفعّال في إطلاق الدواء في مستويات مختلفة من دراجات الحموضة. أفضل التركيبات أظهرت وقت إطلاق يتجاوز الـ8 ساعات. تم اختيار النظام المناسب بناء على دراسات اللزوجة والدراسات البيولوجية.

الكلمات الدالة: نظام مصفوفة الجل الموضعي، كيتوزان، ألجينات الصوديوم، جيلوسير، باراسيتامول، إطلاق مُستدام.

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The Protective Role of *Lannea coromandelica* (Houtt.) Merr. against Histamine Release and Action: Insights from *In vitro, In vivo* Investigations

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ABSTRACT

This study aims to evaluate the antihistaminic potential of the plant extract from *Lannea coromandelica* using both in vitro and in vivo models. In vitro antihistaminic effects were studied using isolated guinea pig ileum to assess dose-dependent inhibitory impacts on histamine-induced contractions. Mast cell density was evaluated using a mast cell count model, calculating the average number of mast cells per unit area in the mesentery. For in vivo assessments, a histamine aerosol-induced bronchospasm model in guinea pigs was used, where pre-convulsive dyspnea (PCD) onset time was noted as pre-convulsive time (PCT). Additionally, a clonidine-induced mast cell degranulation model in rats was employed, with cells stained using 1% toluidine blue to count intact and degranulated mast cells. The *Lannea coromandelica* extract exhibited a dose-dependent inhibition of histamine-induced contractions in isolated guinea pig ileum. Similarly, the extract inhibited mast cell degranulation in a dose-dependent manner, with a higher dose of 400 mg/kg proving more effective than a lower dose of 200 mg/kg. Acute toxicity studies confirmed the safety of the extract at moderate doses, revealing no toxic symptoms at a dosage of 2000 mg/kg body weight. Importantly, the extract significantly increased PCT in guinea pigs and reduced the percentage of disrupted mast cells induced by clonidine. *Lannea coromandelica* shows promising antihistaminic properties, effectively inhibiting histamine-induced bronchospasm and mast cell degranulation, which can be an option for the development of antiasthmatic drugs.

Keywords: *Lannea coromandelica,* antihistamine, guinea pigs, Wistar rats.

1. INTRODUCTION:

Asthma is a chronic inflammation that drives the attention of entire mankind because of its severity in patients [1]. The inflammatory response in asthma involves various immune cells, including mast cells, eosinophils, T lymphocytes, and dendritic cells. These cells release pro-inflammatory mediators, such as histamine, leukotrienes, and cytokines, contributing to airway inflammation and hyperresponsiveness [2][3]. Histamine is a bioactive amine released primarily from mast cells and, to a lesser extent, from other cells such as basophils. Histamine is a key mediator in allergic reactions and induces bronchoconstriction, specifically in asthma patients even at low doses. The airway hyperresponsiveness in asthma causes difficulty in breathing, wheezing, and coughing [4]. Controlling histamine release is crucial in managing asthma, given that histamine plays a significant role in eliciting and exacerbating asthma symptoms. Antihistaminic agents, such as H1 receptor antagonists, serve a supportive role in asthma treatment, particularly for symptoms triggered by allergies. They aid in bronchodilation, similar to β2-stimulants, by blocking histamine's action, thus reducing airway smooth muscle contraction in allergic asthma [5].

Lannea coromandelica is a deciduous tree that belongs

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to the family Anacardiaceae. It is popularly known as the Indian ash tree and is native to South and Southeast Asia [6]. The tree is 10-20 meters tall. Leaves are alternate, opposite, estipulate, and swollen at the base. Flowers are unisexual and yellowish-green; the fruit is a drupe with a hard stone and compressed seeds. It is geographically distributed from Sri Lanka to Southern China [7].

The traditional applications of L. coromandelica include treatment for hepatitis, diabetes, ulcers, heart disease, and dysentery [8]. The aerial parts are enriched with phytochemicals such as anthraquinones, flavonoids like (+)-leucocyanidin, rutin, quercetin, morin, and isoquercitrin; flavonoid glycosides like guaijaverin; and tannins like ellagic acid, which have been isolated from various parts of L. coromandelica. Pharmacologically, L. coromandelica is reported to be effective in controlling glucose levels and has cardioprotective, anti-hyperlipidemic, and diverse pharmacological activities. Its gum exhibits drug delivery potential, and the bark extract demonstrates antibacterial and anticancer effects [9][10][11].

L. coromandelica possesses potent anti-inflammatory and immunomodulatory properties, which could make it a promising natural remedy for asthma [12]. This study aims to address this gap by providing more robust evidence for the therapeutic potential of L. coromandelica as an antihistaminic agent for asthma through in vitro and in vivo studies for the development of new and effective treatments for asthma.

2. MATERIALS AND METHODS

2.1. Plant material and extraction

The leaves of *Lannea coromandelica* (Houtt.) Merr. were collected from the forest areas of Tirupati, Andhra Pradesh, India, in February. The leaves were dried, powdered, and subjected to Soxhlet extraction using ethanol. The solvent was removed, and the percentage yield was calculated using the formula:

% yield= Weight of the extract obtained/ Weight of the raw material × 100

2.1. Preliminary phytochemical analysis

Standard protocols were used to qualitatively identify a variety of phytochemicals present in the ethanolic extract of *L. coromandelica* [13][14][15][16].

2.2. Total flavonoid content

The Dowd colorimetric method using $AlCl_3$ was adapted to determine the total flavonoid content in the ethanolic extract of *L. coromandelica* [17].

2.3. *In vitro* antihistaminic activity

Intestines were dissected from anesthetized guinea pigs, and the distal section was divided into 2-3 cm long pieces. They were placed in a 25 mL organ bath containing Tyrode physiological solution at a constant temperature of 37°C, with a steady tension of 1 g and continuous aeration for 30 minutes. Various concentrations of histamine from a 25 µg/mL stock (0.1, 0.2, 0.4, 0.8, 1.6 mL) were used to identify the submaximal response. The concentration that induced a submaximal response was utilized for subsequent testing. Each test sample, including the standard drug (Mepyramine 0.04 µg/mL) and various concentrations of *L. coromandelica* extract (4 mg/mL and 8 mg/mL), was individually added after a five-minute interval to observe their impact on histamine-mediated contraction. The changes in tissue contraction were accurately recorded using a micro dynamometer [18].

2.4. Mesenteric Mast Cell Count

Adult Wistar rats were anesthetized and euthanized through cervical dislocation. The mesentery was dissected from the abdominal cavity and placed in a petri dish containing a 10% neutral buffered formalin fixative solution. Three batches were made, and Ketotifen (10 µg/mL), and 200 mg/mL and 400 mg/mL concentrations of *L. coromandelica* extract were added respectively and incubated for 15 minutes at 37°C. The mesentery was embedded in paraffin and sliced into thin sections measuring 5-6 microns after fixation. These sections were then stained and observed under a microscope, utilizing resolutions of 40x and 60x, to identify and quantify the

mast cells within a specific unit area of the mesentery. The mast cell count was calculated as the average number of mast cells per unit area of the mesentery [19].

2.5. Acute toxicity studies

The acute toxicity of the test extracts was assessed following the guidelines set by the OECD. The "up and down" method was employed with a limit test conducted at a 2000 mg/kg body weight dosage, using a progression factor of 1.3. The animals were monitored for 14 days, and any deaths occurring within this period were documented [20].

2.6. Histamine-induced bronchospasm in guinea pigs

The histamine-induced bronchospasm in guinea pigs model replicates pathophysiological conditions similar to human asthma, characterized by airway inflammation, mucus secretion, and bronchospasm. Bronchospasm was experimentally induced in guinea pigs by exposing them to histamine aerosol. Guinea pigs of both genders were selected and divided into the following groups (n = 5): Group I - Control, Group II - Ketotifen (10 µg/mL), and Group III and IV - Extracts (Low and High concentrations). The animals displayed progressive difficulty breathing (dyspnea) when exposed to histamine aerosol at a pressure of 40 mmHg from a nebulizer placed in the histamine chamber (manufactured by M/s Inco Ambala, India). The time at which pre-convulsive dyspnea (PCD) began was recorded as the pre-convulsive time (PCT), and as soon as PCD occurred, the animals were immediately transferred to an area with fresh air. On the 5th day, the PCT was measured 2 hours after the last administration of the extracts [21].

The percentage increase in PCT was estimated using the equation:

$$\% PCT = (1 - T1/T2) \times 100$$

Where, T1 = time for PCD onset on day 0 , T2 = time for the PCD onset on day 5

2.7. The Clonidine-induced mast cell degranulation in rats

Wistar rats were divided into four groups, each consisting of five rats. Group I received an oral administration of 5 mL/kg of the vehicle. Group II received an intraperitoneal injection of 50 mg/kg of sodium cromoglycate. Group III and IV were orally administered low and high doses of the *L. coromandelica* extract, respectively. On the seventh day, the peritoneal cavity was injected with 10 mL of normal saline solution, followed by a gentle abdominal massage for 90 seconds, and the fluid containing mast cells was aspirated and collected in a siliconized test tube containing 7-10 mL of RPMI-1640 Medium (pH 7.2-7.4). The mast cell suspension, approximately 1×1061 \times 10^61×106 cells/mL, was then challenged with a 0.5 µg/mL clonidine solution and stained with 1% toluidine blue. Under a high-power microscope, 100 cells were counted from various visual areas, and the number of intact and degranulated cells was recorded [22].

3. RESULTS

3.1. Preliminary phytochemical analysis

The percentage yield was found to be 7.5%, and standard protocols were followed to conduct the preliminary phytochemical analysis of *L. coromandelica*. The analysis revealed the presence of various compounds such as alkaloids, glycosides, flavonoids, terpenoids, steroids, tannins, proteins, carbohydrates, amino acids, and saponins (Table 1).

Table 1. Preliminary phytochemical analysis of *L. coromandelica*

Phytochemicals	***Lannea coromandelica***
Alkaloids	+
Glycosides	+
Flavonoids	++
Terpenoids	++
Steroids	+
Tannins	++
Proteins	++
Carbohydrates	++
Amino acids	+
Saponins	+

+ Present,- Absent

3.2. Total flavonoid content

The total flavonoid content in the plant extract was determined utilizing the colorimetric method with $AlCl_3$. The total flavonoid content of the extract was calculated and represented as milligrams of quercetin equivalents (QE) per gram of dry weight sample (mg/g). The ethanolic extract of *L. coromandelica* has a flavonoid content of 40.7±1.53 mg QE/g under the given conditions and procedures.

3.3. *In vitro* antihistaminic activity

The results in Table 2 demonstrate the effect of *L. coromandelica* on histamine-induced contraction in isolated guinea pig ileum. When treated with *L. coromandelica* at a lower dosage, i.e., 4 mg/mL, the mean contraction reduced to 75.17±2.36 units, significantly lower than the control group, suggesting the ability to inhibit histamine-induced contractions. However, treatment with a higher dosage (8 mg/mL) further decreased the mean contraction to 65.93±1.46 units.

Table 2. Effect of *L. coromandelica* on histamine-induced contraction in isolated guinea pig ileum

Concentration of Histamine	Control group	Mepyramine Group	*L. coromandelica* (4mg/mL)	*L. coromandelica* (8mg/mL)
1.6 µg/mL	91.25±1.27	1.48±1.25**	75.17±2.36*	65.93±1.46**

The significance of the differences between the treated and control groups was determined using One Way ANOVA, followed by Dunnett's test, with $^{***}P<0.001$ and $^{**}P<0.01$, $^{*}P<0.05$ being considered statistically significant.

3.4. Mesenteric Mast Cell Count

The experiment investigated the effect of ethanolic extracts on mast cell degranulation. Ketotifen, a known mast cell stabilizer, shows significant inhibition of mast cell degranulation by 82.61%. *L. coromandelica* at the lower dose shows a 45.18% inhibition of degranulation, which is not statistically significant compared to the control group or Ketotifen. On the other hand, the higher dose of *L. coromandelica* extract (400 mg/mL) shows a 59.37% inhibition of mast cell degranulation, which is higher than the lower dose and is statistically significant compared to the control group (Table 3).

Table 3. Effect of *L. coromandelica* on mast cell degranulation in rats

Treatment	% inhibition of degranulation
Ketotifen	82.61±1.18
L. coromandelica (200mg/ml)	45.18±2.67^{ns}
L. coromandelica (400mg/ml)	59.37±1.64**

The significance of the differences between the treated and control groups was determined using One Way ANOVA, followed by Dunnett's test, with $^{***}P<0.001$ and $^{**}P<0.01$, $^{*}P<0.05$ being considered statistically significant.

3.5. Acute toxicity studies

Acute toxicity studies, performed according to OECD guidelines using the "up and down" method, showed that *L. coromandelica* does not exhibit any toxicity symptoms in rats at a dose of 2000 mg/kg body weight orally. Therefore, it is considered safe to use the extract at medium doses. The working dose was calculated as 1/5th and 1/10th of the highest safe dose, i.e., 400 and 200 mg/kg body weight, respectively.

3.6. The Clonidine-induced mast cell degranulation in rats

The percentage of disrupted mast cells in the control group was 80.36%. The administration of sodium

cromoglycate significantly reduced the percentage of disrupted mast cells to 25.16%. In the *L. coromandelica* treatment groups, the percentage of disrupted mast cells was 71.55% at 200 mg/kg and 63.19% at 400 mg/kg, suggesting that *L. coromandelica*, at a higher dose, has the potential to reduce mast cell degranulation (Table 4).

Table 4. Effect of *L. coromandelica* on Clonidine-induced mast cell degranulation in rats

Treatment	% of disrupted mast cells
Control	80.36±1.15
Sodium cromoglycate	25.16±0.38
L. coromandelica (200mg/kg)	71.55±1.18^{ns}
L. coromandelica (400mg/kg)	63.19±1.34**

The significance of the differences between the treated and control groups was determined using One Way ANOVA, followed by Dunnett's test, with $^{***}P<0.001$ and $^{**}P<0.01$, $^{*}P<0.05$ being considered statistically significant.

3.7. Histamine-induced bronchospasm in Guinea pigs

The effects of various extracts on the pre-convulsive time in guinea pigs were investigated. Ketotifen at a concentration of 10 µg/mL significantly increased pre-convulsive time from 101.77±6.94 on Day 0 to 146.55±10 on Day 5.

L. coromandelica, at a dose of 200 mg/kg, did not exhibit a statistically significant effect on pre-convulsive time. However, when the dose was increased to 400 mg/kg, it showed a significant effect with a pre-convulsive time that increased from 104.86±7.15 on Day 0 to 116.02±7.92 on Day 5. This indicates that the higher dose of *L. coromandelica* significantly prolonged the pre-convulsive time in guinea pigs (Table 5).

Table 5. Effect of *L. coromandelica* on pre-convulsive time in Guinea pigs

Treatment	Day 0	Day 5
Control	93.55±6.38	94.48±6.45
Ketotifen (10 µg/ml)	101.77±6.94****	146.55±10****
L. coromandelica (200mg/kg)	97.66±6.66^{ns}	99.71±6.8^{ns}
L. coromandelica (400mg/kg)	104.86±7.15^{ns}	116.02±7.92***

The significance of the differences between the treated and control groups was determined using One Way ANOVA, followed by Dunnett's test, with $^{***}P<0.001$ and $^{**}P<0.01$, $^{*}P<0.05$ being considered statistically significant.

4. DISCUSSION

The in vitro antiasthmatic activity data demonstrated that *L. coromandelica* mitigated histamine-induced contractions in isolated guinea pig ileum. There was a marked difference between the control group, which displayed a mean contraction, and the standard group, which demonstrated significantly lesser contraction, highlighting pronounced inhibition of histamine-induced contractions. Treatment with *L. coromandelica* at a dosage of 200 mg/kg significantly reduced the mean contraction, indicating a notable decrease compared to the control group. Interestingly, increasing the dosage to 400 mg/kg further reduced the mean contraction, which was statistically significant compared to both the control group and the lower dosage groups. These results suggest a dose-dependent inhibitory effect of *L. coromandelica* against histamine-induced contractions, presenting potential therapeutic benefits in managing asthmatic manifestations. Our findings align with similar

experiments on *Mirabilis jalapa*, which support its traditional applications [23].

The investigation into the effects of *L. coromandelica* extracts on mast cell degranulation revealed that at a lower dose of 200 mg/kg, there was no statistically significant inhibition of mast cell degranulation compared to both the control group and Ketotifen. However, at 400 mg/kg, the extract not only exceeded the inhibitory performance of its lower-dose counterpart but also demonstrated statistically significant mitigation of mast cell degranulation compared to the control group.

In the context of acute toxicity studies, *L. coromandelica* manifested no symptoms of toxicity in rats even at a substantial dose of 2000 mg/kg body weight when administered orally, indicating its safety in medium-dose applications. This finding correlates with similar observations reported by Venkatesham et al., 2019 [24]. The significant reduction in mast cell disruption observed in the *L. coromandelica* treatment groups during clonidine-induced mast cell degranulation reinforces the extract's impact in physiological contexts relevant to mast cell activity and stability. These results are consistent with the research conducted by Kumar et al., 2010, who also reported significant protection against mast cell degranulation when challenged with clonidine, indicating mast cell stabilizing activity [25].

Additionally, phytochemical analyses elucidated that *L. coromandelica* is rich in flavonoids, a class of secondary metabolites often associated with various biological activities, including anti-inflammatory and antioxidant properties. The abundant flavonoid content not only enhances the pharmacological potential of *L. coromandelica* but also potentially elucidates the mechanistic pathways through which the extract exerts its effects on mast cell stabilization and inhibition of degranulation [26].

5. SUMMARY AND CONCLUSION

In vitro studies demonstrated that L. coromandelica extract possesses significant antihistaminic activity by effectively inhibiting histamine-induced contractions in isolated guinea pig ileum. This inhibitory effect showed a dose-dependent response, particularly at the higher dosage of 400 mg/kg. Investigations involving clonidine-induced mast cell degranulation in rats underscored the potential of *L. coromandelica* as an antihistaminic agent by inhibiting clonidine-induced mast cell degranulation. Additionally, histamine-induced bronchospasm experiments in guinea pigs indicated that *L. coromandelica* extract exerted a protective effect against convulsions, as reflected by a slight increase in pre-convulsive time on Day 5.

In conclusion, the in vitro antihistaminic activity of *L. coromandelica* demonstrated its ability to inhibit histamine-induced contractions in isolated guinea pig ileum. The higher dosage of 400 mg/kg exhibited a stronger inhibitory effect compared to the lower dosage of 200 mg/kg, suggesting a dose-dependent response. Furthermore, *L. coromandelica* extract displayed inhibitory effects on mast cell degranulation, particularly at the higher dosage of 400 mg/kg, indicating its potential as a stabilizer. Thus, the results collectively advocate *L. coromandelica* as a candidate worthy of further research and development in the context of therapies involving mast cell stabilization and related antiasthmatic applications.

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Conflict of interest

The authors declare no conflict of interest.

Authors contribution

All the authors contributed to the conception, and design and approved the submission.

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الدور الوقائي للانيا كورومانديليكا (هوت.) مير. ضد إفراز وعمل الهستامين: رؤى من التحقيقات في المختبر وفي الجسم الحي

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ملخص

تهدف هذه الدراسة إلى تقييم القدرة المضادة للهستامين للمستخلص النباتي منلانيا كوروماندليكا باستخدام كليهما في المختبر ونماذج على قيد الحياة. في المختبر تمت دراسة التأثيرات المضادة للهستامين باستخدام اللفائفي المعزول لخنزير غينيا لتقييم التأثيرات المثبطة المعتمدة على الجرعة على الانقباضات الناجمة عن الهستامين. تم تقييم كثافة الخلايا البدينة باستخدام نموذج عدد الخلايا البدينة، وحساب متوسط عدد الخلايا البدينة لكل وحدة مساحة في المساريق. لعلى قيد الحياة التقييمات، تم استخدام تشنج قصبي الهستامين الناجم عن الهباء الجوي في نموذج خنزير غينيا، حيث لوحظ أن وقت بداية ضيق التنفس قبل التشنج (PCD) هو وقت ما قبل التشنج .(PCT) بالإضافة إلى ذلك، تم استخدام نموذج تحلل الخلايا البدينة الناجم عن الكلونيدين في الفئران، مع تلوين الخلايا باستخدام 1% من التولويدين الأزرق لحساب الخلايا البدينة السليمة والمحببة. اللانيا كوروماندليكا أظهر المستخلص تثبيطًا يعتمد على الجرعة للتقلصات التي يسببها الهستامين في اللفائفي لخنزير غينيا المعزول. وبالمثل، فإن المستخلص يمنع تحلل الخلايا البدينة بطريقة تعتمد على الجرعة، حيث أثبتت جرعة أعلى قدرها 400 ملجم / كجم أنها أكثر فعالية من جرعة أقل قدرها 200 ملجم / كجم. أكدت دراسات السمية الحادة سلامة المستخلص عند تناول جرعات معتدلة، ولم تظهر أي أعراض سمية عند جرعة 2000 ملجم/كجم من وزن الجسم. الأهم من ذلك، أن المستخلص زاد بشكل كبير من معاهدة التعاون بشأن البراءات في خنازير غينيا وخفض النسبة المئوية للخلايا البدينة المعطلة الناجمة عن الكلونيدين. لانيا كوروماندليكا يُظهر خصائص واعدة مضادة للهيستامين، حيث يثبط بشكل فعال التشنج القصبي الناجم عن الهيستامين وتحلل الخلايا البدينة التي يمكن أن تكون خيارًا لتطوير الأدوية المضادة للربو.

الكلمات الدالة: لانيا كوروماندليكا، مضادات الهيستامين، خنازير غينيا، فئران ويستار.

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A Review of Safety, Quality, Regulation, and Delivery Approaches for Phytopharmaceuticals

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ABSTRACT

Phytopharmaceuticals are plant-derived compounds with a wide range of potential health benefits. Their unique characteristics and versatile applications make them promising candidates for the treatment of many diseases. Phytopharmaceuticals contain a wide range of bioactive components, including alkaloids, terpenes, and flavonoids. These compounds have a variety of biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects. In recent years, there has been growing interest in phytopharmaceuticals for the treatment of various conditions, including cancer, cardiovascular disease, and diabetes. However, more research is needed to fully understand the efficacy and safety of these compounds. To ensure the quality and safety of phytopharmaceuticals, quality management procedures have been developed based on the principles of the World Health Organization (WHO) and Good Agricultural and Collection Practices (GACP). Understanding these regulations is essential for assuring effective phytopharmaceutical product development, manufacture, and distribution. While phytopharmaceuticals have shown promise in laboratory research, there are challenges in translating their efficacy to effective clinical applications, particularly in terms of delivery. Innovative approaches, such as targeted delivery methods and nanoparticle-based strategies, are needed to overcome these challenges. This review provides a comprehensive overview of the challenges and opportunities in the field of phytopharmaceuticals.

Keywords: Phytopharmaceuticals, Quality, Regulations, Challenges, FDA approved phytopharmaceuticals.

1. INTRODUCTION

From ancient times, humans have harnessed the power of natural products, with animals, plants, and minerals serving as key sources of medicinal compounds. Traditional remedies form an essential component of phytomedicine, recognized as phytopharmaceuticals. According to the World Health Organization, in the majority of developing countries, 70% to 95% of individuals primarily rely on traditional medicine for healthcare. Herbal medicinal products, commonly referred to as phytopharmaceuticals, are pharmaceutical preparations made from plant materials. They have been utilized by various cultures for centuries to treat a wide range of health conditions, harnessing the medicinal properties of different plant parts such as leaves, roots, stems, flowers, and fruits to formulate remedies in various forms like teas, tinctures, extracts, capsules, and powders, owing to the therapeutic effects of the active compounds found in these plants (1).

The terms "phytopharmaceutical" or "phytopharmakon" originate from Greek words. "Phyto" is derived from "phyton," which means "plant," and "pharmakon" means "medicine." A phytopharmaceutical drug is defined as a purified and standardized portion of an extract of a medicinal plant or its part containing at least

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four bioactive or phytochemical compounds (qualitatively and quantitatively assessed) for internal or external use by humans or animals for the diagnosis, treatment, mitigation, or prevention of any disease or disorder, but does not include parenteral administration (2).

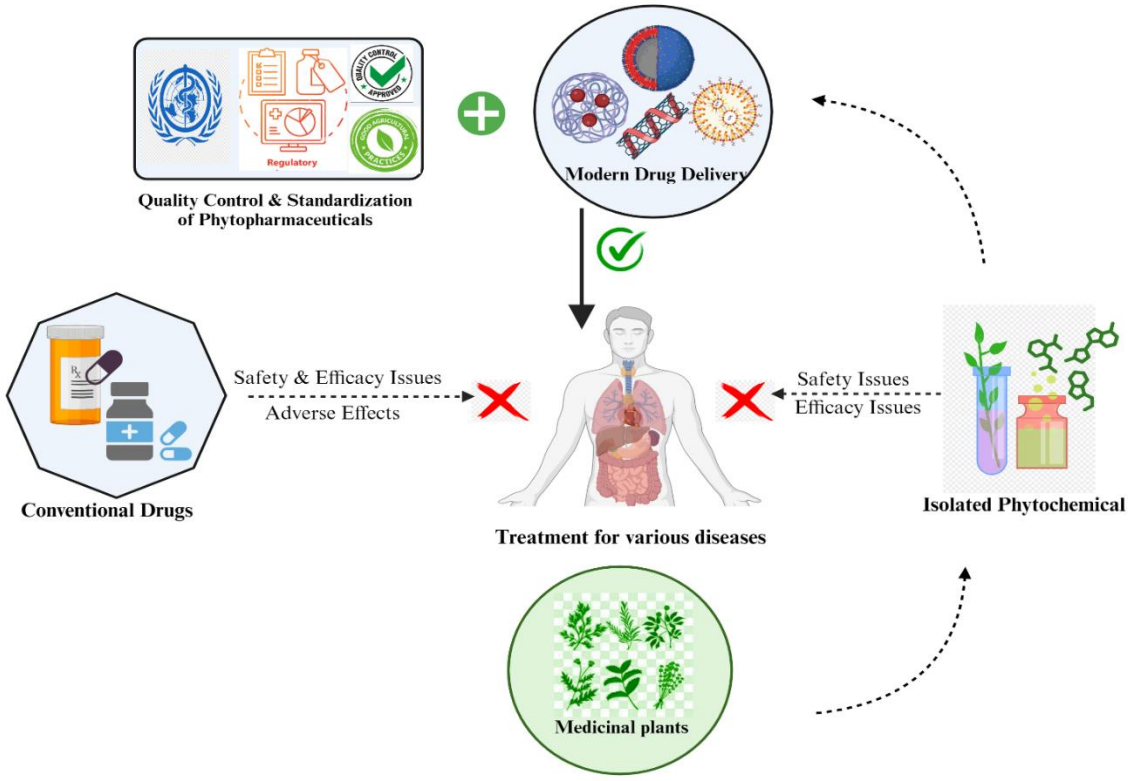


Fig: 1- Development of plant derived medicine by integration of Modern drug delivery methods and Quality control process to enhance the Safety and efficacy of phytopharmaceuticals

The term "phytopharmaceuticals" highlights the link between plants and their potential to offer medicinal advantages. This concept reflects the historical and ongoing utilization of plants within traditional and modern healthcare systems. Phytopharmaceuticals contain a diverse array of bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolic compounds, which contribute to their therapeutic effects. These compounds can exert a wide range of physiological actions, such as anti-inflammatory, antioxidant, antimicrobial, analgesic, immune-modulating, and hormone-regulating activities. In recent years, there has been renewed interest in phytopharmaceuticals due to a growing demand for alternative and complementary therapies, as well as an increased focus on holistic and personalized healthcare approaches (3).

Scientific research is being conducted to validate the traditional uses of these plant-based medicines and to understand their mechanisms of action. The use of phytopharmaceuticals is deeply rooted in traditional medicine systems like Ayurveda, Traditional Chinese Medicine (TCM), and Indigenous knowledge, where plant-based remedies have been employed to maintain health and manage diseases.

Phytopharmaceutical products effectively manage health issues using a combination of active compounds (Fig-1). Unlike conventional drugs that target singular biological targets, phytopharmaceuticals leverage groups of molecules to address multiple targets simultaneously. This is why these products commonly contain various ingredients, maximizing their combined potential for intended effects (4).

2. QUALITY, SAFETY AND EFFICACY OF PHYTOPHARMACEUTICALS

In addition to bioavailability, two other crucial attributes of phytopharmaceuticals are their efficacy and safety. Efficacy pertains to the attainable response exhibited by a cell, organ, organism, or animal when exposed to a pharmaceutical agent. Safety, on the other hand, gauges the absence of adverse events associated with the agent's use (Fig-2).

2.1. Safety

Safety is important in the use of phytopharmaceuticals as they are derived from plants that are not automatically safe and free from adverse effects. Plants contain hundreds of chemical compounds, some of which can have potent and toxic effects. For example, certain anti-cancer agents, digitalis compounds, and pyrrolizidine alkaloids found in plants can be highly toxic. It is an erroneous assumption that phytomedicines are inherently safe without any side effects. Rigorous safety testing of phytopharmaceuticals is crucial to identify any toxic phytochemicals, potential drug interactions, or adverse reactions they may cause. This involves assessing the potential for adverse effects or harm when these products are administered. Adverse effects can range from mild discomfort to severe reactions. Regulatory agencies worldwide set stringent standards for safety evaluation. Comprehensive toxicity studies, clinical trials with placebo controls, and post-marketing surveillance contribute to assessing the safety profile of phytopharmaceuticals.

Safety considerations encompass several factors, including dosage, duration of exposure, molecular composition, reactivity, and the presence of coexisting medical conditions. These elements, along with other potential variables, collectively influence the safety profile of a substance during its consumption (5,6).

For instance, Belladonna contains toxic alkaloids like atropine and scopolamine that can cause anticholinergic toxicity at high doses. However, recent toxicity studies have helped determine lethal doses and characterize side effects, establishing dosage guidelines and parameters for its safe use. A randomized controlled trial showed that topical belladonna plaster was well tolerated short-term at low doses of 3 mg atropine and 0.2 mg scopolamine. Another study found that sublingual atropine eyedrops derived from belladonna were safe at doses under 1 mg. These studies demonstrate that belladonna can be used safely when administered at controlled low doses, avoided systemically, and with monitoring for adverse effects. Further research will continue to refine the safety profile and parameters for the medicinal use of belladonna (7).

Recent studies show that colchicine may be effective for treating cardiovascular diseases at low doses (0.5-1.0 mg/day), with reduced adverse effects compared to higher doses. More research is needed to optimize dosing and further define colchicine's efficacy and safety profile for cardiovascular treatment (8).

2.2. Efficacy

The efficacy of phytopharmaceuticals refers to their ability to produce the desired therapeutic effects. This effectiveness is influenced by various factors, including the specific bioactive compounds present in the plant, their concentrations, and how they interact with the body's biological processes. Rigorous scientific studies, including preclinical and clinical trials, are conducted to determine the efficacy of phytopharmaceuticals. These trials help establish the optimal dosages, routes of administration, and expected outcomes when these products are used to

treat specific medical conditions.

2.2.1. ***In-vitro studies:*** These investigations involve controlled laboratory conditions to evaluate the impact of these natural compounds on diverse biological systems. Various assays are employed, including tests to gauge cell viability and cytotoxicity, assess antimicrobial properties against bacteria and fungi, evaluate antioxidant potential, study enzyme inhibition, examine cell signaling pathways, analyze apoptosis and cell cycle effects, and investigate potential neuroprotective and wound healing properties. These studies provide valuable insights into the potential therapeutic benefits of phytopharmaceuticals, aiding in the identification of promising candidates for further validation through in vivo experiments (9).

An in vitro study examined the antioxidant and anti-inflammatory properties of curcumin, a phyto compound found in turmeric. The results demonstrated that curcumin exhibited significant antioxidant activity, scavenging free radicals, and anti-inflammatory activity by inhibiting the production of inflammatory mediators. These findings support the potential therapeutic benefits of curcumin in various conditions associated with oxidative stress and inflammation (10).

2.2.2. ***In-vivo studies:*** This involves the examination of interventions within living organisms or whole systems, rather than in controlled lab settings. For assessing the efficacy of phytopharmaceuticals, these studies encompass a range of approaches, including trials with animal models to gauge physiological responses, toxicity, and disease models. Additionally, human clinical trials provide essential insights into safety, dosage, and therapeutic effects. Such research delves into factors like pharmacokinetics, metabolism, bioavailability, and potential interactions, shedding light on the compounds' mechanisms of action and long-term consequences. In vivo studies offer a comprehensive understanding of phytopharmaceuticals' effects within the intricate landscapes of living organisms, complementing data obtained from laboratory-based investigations (6,11).

A randomized, double-blind, placebo-controlled trial evaluated the efficacy and safety of silymarin, a phyto compound derived from milk thistle, for the treatment of non-alcoholic fatty liver disease (NAFLD). The study found that silymarin significantly improved liver function markers, reduced inflammation, and was well-tolerated by patients with NAFLD (12).

2.3. Quality

Phytopharmaceuticals need to undergo rigorous quality control testing to ensure their safety, identity, purity, and potency. This involves various analytical techniques to verify that the correct plant species is being used and to identify and quantify the active compounds. The safety and effectiveness of phytopharmaceuticals are directly impacted by the quality control measures applied to medicinal plants within herbal products. Presently, chemical analysis stands out as the most efficient means of standardization, detecting contaminants, and verifying the identity of medicinal plants. In addition to other approaches, molecular biology-based techniques can complement these methodologies for medicinal plant authentication (13).

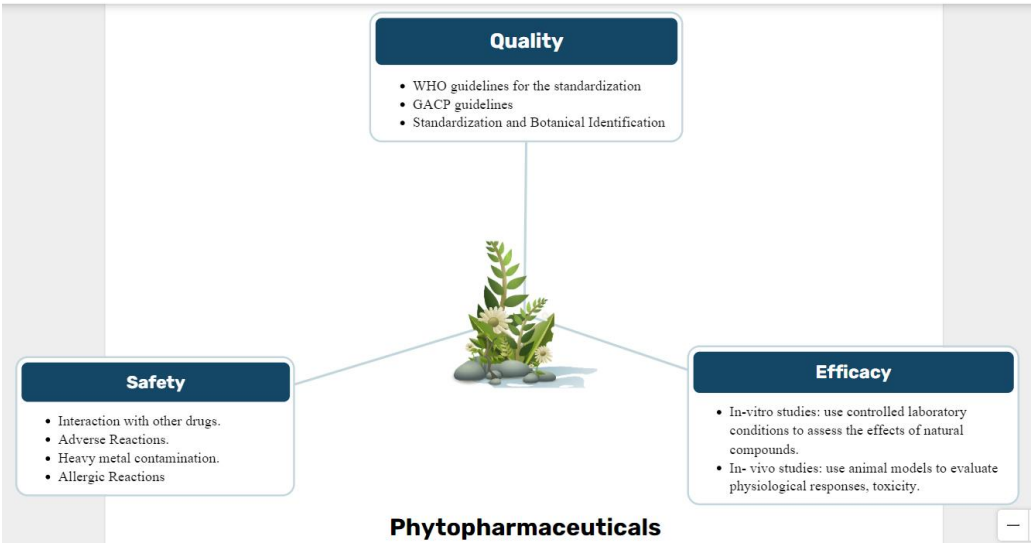


Fig: 2- Quality, safety and efficacy of a phytopharmaceuticals

2.3.1. WHO recommendations for standardising unprocessed herbal medicine ingredients (14)

Table:1- WHO Recommendations for Quality Control Testing of Unprocessed Herbal Medicine Ingredients

Quality control Parameter	Recommendation
Authentication	Verify stage of collection, plant parts, origin, botanic identity
Foreign matter	Should be free of contaminants like soil, insects, animal waste
Organoleptic evaluation	Assess appearance, texture, colour, odour, taste
Tissues of Diagnostic Importance	Verify presence of key anatomical features for Authenticity.
Ash values	Determine total ash, acid-insoluble ash
Extractive values	Determine water-soluble and alcohol-soluble extracts
Volatile matter	Determine the percentage of volatile compounds in the herbal material.
Moisture content	Specific moisture content to assess storage and microbial risks.
Chromatography/spectroscopic Evaluation	Verify identity, purity, chemical markers
Heavy metals Determination	Analyse for heavy metals such as cadmium, lead, arsenic, mercury
Pesticide residues	Check for residues of pesticides or herbicides used during cultivation.
Microbial contamination	Assess the presence of bacteria, moulds, yeast, and other microorganisms.
Aflatoxins	Should be absent or within specified limits
Radioactive contamination	Specify limits for radioactive contaminants

2.3.2. GACP guidelines

The WHO has established essential technical standards to regulate the quality of bioactive compounds (Table-1). Among these standards, the latest guidelines pertain to Good Agricultural and Collection Practices (GACP) for medicinal plants. This involves guidelines and practices that ensure appropriate agricultural practices, collection, and harvesting of medicinal plants in a sustainable and environmentally friendly manner. These guidelines are designed to improve the effectiveness, safety, and overall quality of the finalized herbal products by ensuring uniform levels of active compounds, minimizing risks of contamination or adulteration, and aligning with regulatory standards to enhance the overall safety of the end product.

Additionally, GACP guidelines extend their influence to related standard operating procedures. Collaborative efforts are encouraged to facilitate the development of these guidelines, promoting a collective approach to their refinement and implementation. This approach includes aspects beyond quality control and safety assurance (15).

2.3.3. Standardization and Botanical Identification

Ensuring the accurate identification of plant species is vital for both the effectiveness of the end product and consumer safety. Botanical identification primarily focuses on accurately identifying the plant species used in the production of phytopharmaceuticals. It involves techniques such as DNA barcoding, microscopic analysis, and other botanical authentication methods to ensure that the correct plant species is being used, which is crucial for product efficacy and consumer safety (16).

On the other hand, standardization involves ensuring consistent and reproducible quality in the final product by controlling the levels of specific active compounds or markers that contribute to the product's therapeutic effects. By employing analytical techniques such as high-performance liquid chromatography (HPLC) or gas chromatography (GC), these compounds are quantified, ensuring a consistent chemical profile. Standardization helps guarantee that consumers receive the same level of efficacy and benefits with each use of the product. Combining traditional medicine with modern science, standardization ensures that phytopharmaceuticals offer reliable and predictable therapeutic benefits to consumers (17).

2.3.4. Heavy Metal Analysis and Microbial testing

Heavy metal analysis is critically important in the context of phytopharmaceuticals and other herbal products due to the potential health risks associated with heavy metal contamination. To mitigate this risk, heavy metal analysis employs accurate techniques such as atomic absorption spectroscopy or inductively coupled plasma mass spectrometry. These techniques allow manufacturers to quantify the levels of heavy metal contaminants present in the plant material. Regulatory bodies often set maximum permissible limits for heavy metal content in phytodrugs, ensuring consumer safety. Medicinal plants may contain various microorganisms, including bacteria, yeast, and molds. Microbiological testing ensures that the products are within acceptable limits for microbial contamination (18,19).

2.3.5. Processing and Storage

During processing, the harvested or collected plant material undergoes meticulous cleaning, sorting, and extraction processes to yield bioactive compounds. Techniques such as cutting, grinding, and concentrating are employed to optimize extraction efficiency. However, the integrity of these compounds heavily depends on the subsequent storage conditions. Adequate storage is essential to prevent degradation and contamination (20). Factors such as temperature, humidity, light exposure, and airtight packaging play a critical role in maintaining the

potency of active compounds and deterring the growth of contaminants. By implementing proper processing techniques and ensuring optimal storage environments, producers safeguard the therapeutic potential and safety of the final herbal medicine products, promoting consumer confidence and fostering the effectiveness of botanical remedies (13).

3. APPLICATIONS OF PHYTOPHARMACEUTICALS (21,22)

Many natural compounds from plants act as phytopharmaceuticals by exerting specific health effects in the body. These natural compounds serve as the foundation for a wide range of pharmaceutical drugs, herbal supplements, and traditional remedies, harnessing their therapeutic properties to treat various ailments. Additionally, phytopharmaceuticals contribute significantly to the development of novel drugs, promoting research into natural compounds for their potential in combating diseases.

Each phytopharmaceutical has its own pharmacological effects. For instance, flavonoids like quercetin found in fruits and vegetables act as antioxidants, anti-inflammatories, and antimicrobials. Carotenoids including beta-carotene and lutein in carrots and tomatoes protect vision, boost immunity, and reduce cancer risk. Limonoids in citrus demonstrate anticancer effects. Resveratrol from grape skins provides cardio-protection, anti-aging, and neuroprotective benefits. Soy phytoestrogens can aid hormone balance and menopause symptoms. Lycopene-rich fruits offer antioxidant cardiovascular and anticancer activities. Curcumin, gingerol, echinacea, garlic, ginseng, milk thistle, cranberry, and aloe vera gel have specific anti-inflammatory, antioxidant, immune-boosting, antimicrobial, liver-protective, and wound-healing properties. Cinnamon may improve blood sugar control and insulin sensitivity. Table-2 provides an overview of some major categories of bioactive plant-derived compounds along with their dietary sources and evidence-based therapeutic uses. It summarizes the diverse health benefits and pharmacological effects exhibited by flavonoids, carotenoids, phytoestrogens, terpenoids, and other phytonutrients found in fruits, vegetables, herbs, and other botanical sources.

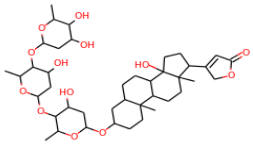
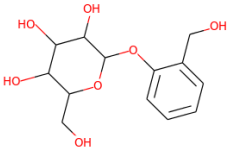
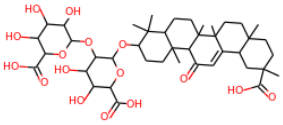
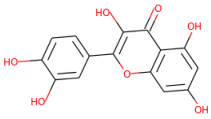
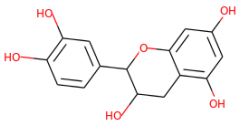
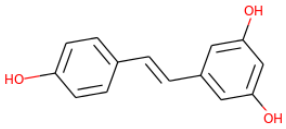
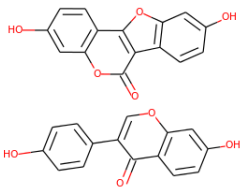
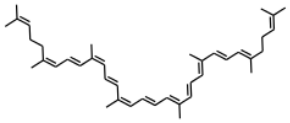
4. CHALLENGES IN DELIVERY OF PHYTOPHARMACEUTICALS

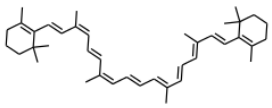
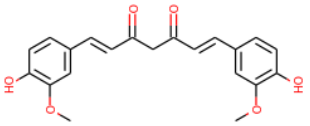
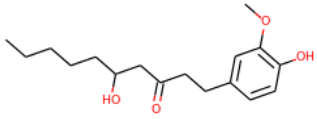
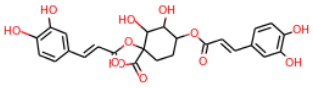
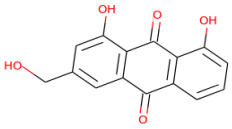
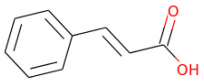
In order to ensure consistent dosing, optimal patient compatibility, stability, and the most effective therapeutic outcomes, it is essential to integrate the herbal drug into an appropriate formulation. Extensive research and development efforts have focused on effectively incorporating phytoconstituents through exploring a diverse range of established and innovative formulation methods.

5.1 Challenges Associated with Medicinal Plants

The challenges concerning medicinal plants include a range of issues involving authentication and quality control, complicated phytochemistry, limited scientific evidence, challenges in standardization, regulatory complexities, concerns regarding sustainability, and considerations related to cultural aspects. These challenges are further compounded by factors such as adulteration and bioavailability. Addressing these concerns requires implementing a comprehensive approach that incorporates both traditional knowledge and modern scientific advancements, while simultaneously prioritizing factors such as quality, sustainability, and adherence to regulatory standards (37).

Table: 2- Overview of Phytopharmaceuticals and their Therapeutic Applications

Active Compounds	Molecular Structure	Sources	Potential Applications
Digitoxin(24)		Digitalis purpurea	Cardioprotective
Salicin(25)		Willow bark	Anti-inflammatory agent
Glycyrrhizin(26)		Glycyrrhiza glabra	Anti-inflammatory, Anti-viral
Quercetin(27)		Fruits, onions, citrus fruits, wine.	Antioxidant, anti-inflammatory, antimicrobial, anticancer
Epicatechin(28)		Dark chocolate, green tea, and berries	Antioxidant, cardioprotective, Antidiabetic
Resveratrol (29)		Grapes, berries, red wine	Anti-aging, anticancer, cardioprotective, neuroprotective
Phytoestrogens (30)		Soy, flaxseeds, legumes	Hormone balance, menopause symptoms, bone health
Lycopene (31)		Tomatoes, watermelon, pink grapefruit	Antioxidant, cardiovascular and anticancer benefits

Active Compounds	Molecular Structure	Sources	Potential Applications
Beta-Carotene(32)		Carrots, Sweet potatoes, Pumpkin	Antioxidant, Vision health, Immune booster.
Curcumin(33)		Turmeric	Anti-inflammatory, antioxidant, anticancer, neuroprotective
Gingerol(34)		Ginger	Anti-inflammatory, nausea relief, digestive aid
Echinacea(35)		Echinacea purpurea	Immune boosting, cold and flu relief
Aloe emodin (36)		Aloe vera leaf gel	Skin health, wound healing, anti-inflammatory
Cinnamic acid (30)		Cinnamon	Hypoglycaemic, Insulin sensitivity

5.2 Pharmaceutical challenges

In the pharmaceutical industry, challenges include low solubility in water and lipids, which affects bioavailability. Complexities in formulation and development, preformulation issues, shelf-life determination, instability, and pharmacokinetic changes also add to the complication. Addressing these issues necessitates multifaceted approaches such as solubility enhancement, precise formulation, stability testing, and careful consideration of pharmacokinetics. By addressing these issues, formulation researchers can ensure that phytopharmaceutical products are delivered effectively (38).

5.2.1 Preformulation Challenges: The primary objective of a preformulation study is to evaluate the physicochemical characteristics of natural products to gain insights into their influence on the development of efficacious dosage forms. This research involves assessing stability, solubility through determination of pKa, pH solubility profile, partition coefficient, and dissolution. When examining herbal formulations that utilize multicomponent extracts, difficulties arise in investigating each individual constituent. Alternatively, researchers have the option to conduct preformulation investigations on the entirety of the plant extract, evaluating a wide array

of factors to achieve the most effective formulation development (39).

5.2.2 Formulation Development Challenges: The formulation and development of herbal extracts face difficulties due to their unique characteristics. These extracts typically exhibit hygroscopic properties, viscosity, and an amorphous structure, resulting in limited flowability and suboptimal physico-mechanical characteristics (40). Controlling and processing specific extracts can be challenging due to their sticky characteristics and tendency to absorb moisture. Another challenge is selecting an appropriate solvent to solubilize both the extract and excipient during formulation. Despite these challenges, viable solutions can be identified to enhance the formulation and advancement of herbal products (41).

5.2.3 Bioavailability issues: Poor aqueous or lipid solubility and bioavailability are major concerns in pharmaceutical development. Compounds with low solubility dissolve poorly in bodily fluids, reducing absorption and efficacy. Lipophilic substances struggle to penetrate cell membranes and reach their targets. Techniques such as micronization, nanosizing, and complexation improve solubility and increase surface area to overcome these challenges. Additionally, lipid-based delivery systems and prodrugs are used to enhance solubilization. Researchers can enhance the therapeutic potential of pharmaceutical compounds by integrating formulation innovation, pharmacokinetics, and drug design to mitigate poor solubility.

5.2.4 Instability: Chemical, physical, and microbiological changes render phytopharmaceuticals unstable. Chemical degradation from light, heat, and moisture reduces efficacy. Physical changes affect appearance and texture, while microbial contamination poses safety risks. Stability testing and optimizing formulations, packaging, and storage conditions are essential to address instability. Botanical and pharmaceutical expertise ensures the potency, quality, and safety of phytopharmaceuticals (3,42).

5. OVERCOMING CHALLENGES THROUGH NOVEL DELIVERY SYSTEMS

Recently, there has been an increase in the usage of novel formulations that incorporate plant extracts or isolated fractions. These compounds are combined with materials possessing various properties to enhance their bioavailability and medicinal effects. Examples of novel formulations include liposomes, solid dispersions, phytosomes, and other morphologies. These herbal formulations offer advantages such as enhanced solubility, targeted distribution, fewer side effects, and controlled release of medicine. However, challenges such as scaling up manufacturing and stability concerns accompany these formulations. Innovative drug delivery methods have the potential to streamline the complex process of delivering phytopharmaceuticals within the pharmaceutical industry (43). This potential can be realized through the following methods:

i. Targeted drug delivery: By leveraging advanced principles of nanotechnology, specific delivery systems can be developed. These systems incorporate specific ligands into formulations to ensure targeted delivery of phytopharmaceuticals to desired sites throughout the body. This precision not only reduces inefficiencies but also enhances therapeutic efficacy by accurately delivering bioactive substances to critical regions (44).

ii. Nanoparticle-Based Delivery: Nano-sized drug carriers such as liposomes, nanoparticles, and micelles revolutionize the delivery of phytopharmaceuticals. These tiny carriers encapsulate compounds, protecting them from degradation and improving bioavailability. Additionally, they enable controlled release over extended periods, maintaining consistent therapeutic levels and reducing dosing frequency (45).

iii. Transdermal and Topical Delivery: Innovative formulations enable phytopharmaceuticals to be delivered through the skin directly into the bloodstream. Transdermal patches, gels, creams, and ointments provide

non-invasive, patient-friendly administration methods. This approach bypasses the gastrointestinal tract, ensuring sustained release and avoiding rapid fluctuations in plasma levels (46).

iv. Intracellular Delivery: Advanced delivery systems address the challenge of penetrating cell membranes to facilitate intracellular delivery of phytopharmaceuticals. This capability is crucial for diseases requiring action within cells, such as certain cancers. Techniques like cell-penetrating peptides and nanocarriers effectively transport bioactive compounds across cell barriers (47).

v. Gastrointestinal Tract Stability: Many phytopharmaceuticals degrade in the harsh acidic environment of the gastrointestinal tract. Novel delivery systems protect these compounds, ensuring intact delivery to the desired site of action. Coatings, matrices, or encapsulation within gastro-resistant formulations enhance stability and bioavailability.

vi. Combination Therapies: Innovative delivery methods enable the combination of multiple phytopharmaceuticals or conventional drugs in a single formulation. This synergistic approach enhances therapeutic outcomes, reduces side effects, and simplifies dosing regimens for patients (48).

6. REGULATORY ASPECT OF PHYTOPHARMACEUTICALS

Phytopharmaceuticals have varying legal statuses in different jurisdictions. Regulation is essential to maintain their safety and credibility. While phytopharmaceuticals are well-recognized in some countries, in others they are considered as food, and making therapeutic claims about them is illegal. Users of these products are at risk because inadequate oversight can lead to harmful overconsumption or insufficient intake of vital nutritional and therapeutic components. This situation exacerbates public health concerns, including worries about the emergence of drug resistance (49).

Governments and organizations are committed to promoting responsible and ethical usage, as demonstrated by their diligent efforts to regulate phytopharmaceutical products. Leading bodies such as the Codex Alimentarius Commission, the WHO, and UNICEF play pivotal roles not only in supervising processing and product standards but also in formulating guidelines for industrial production. Additional entities like Médecins Sans Frontières (MSF) have also played significant roles, especially in the context of ready-to-use therapeutic foods. Their proactive engagement underscores a steadfast commitment to preserving the safety and authenticity of these products.

It is evident that governments are increasingly incorporating phytopharmaceutical products into their regulatory and monitoring frameworks. The USFDA continues to be a prominent global regulatory authority, recently expanding its oversight to include standards for supplement approval. Similar distinct regulatory standards exist at both individual state and regional levels, including within the EU (European Union), SADC (Southern African Development Community), and EAC (East African Community) (50).

7.1 Regulation of Phytopharmaceuticals in India

Regulating phytopharmaceuticals, which are medicinal products derived from plant sources, is critical for ensuring their safety, efficacy, and quality in the Indian pharmaceutical market. The regulatory process involves evaluating the safety profile of phytopharmaceuticals through comprehensive toxicity studies, placebo-controlled clinical trials, and post-marketing surveillance. This process helps identify potential adverse effects, interactions with other medications, and concerns related to dosage.

India's regulatory framework for phytodrugs involves several key entities and guidelines overseeing

their development, production, and distribution (51). The Central Drugs Standard Control Organization (CDSCO) under the Ministry of Health and Family Welfare serves as the central authority overseeing and regulating all pharmaceuticals, including plant-derived medicines. CDSCO evaluates the safety, efficacy, and quality of phytoactive compounds through a structured approval process before market authorization.

Phytopharmaceutical Reference Standards (PPRS) provide benchmarks to validate the quality, purity, and potency of plant-derived active compounds during production and testing, ensuring consistent product specifications are met. Good Manufacturing Practices (GMP) enforce rigorous standards for facilities, equipment, documentation, quality control, and environmental conditions to maintain batch integrity and prevent contamination. Adherence to GMP is a regulatory requirement, emphasizing its role in safeguarding consumer safety.

Together, CDSCO oversight, PPRS standards, and GMP compliance enable India to effectively regulate phytodrugs, upholding their quality, efficacy, and safety.

8. FDA- APPROVED PHYTOPHARMACEUTICALS

The FDA regulates some phytopharmaceuticals as prescription drugs, while others are classified as dietary supplements and are subject to less stringent regulation. Prescription phytopharmaceuticals have demonstrated safety and effectiveness through clinical trials, whereas dietary supplements have not undergone the same rigorous testing. The FDA has approved a limited number of phytopharmaceuticals for use in the United States. Table-3 lists some of the FDA-approved phytopharmaceuticals, their plant sources, and approved uses (52–54).

Table. 3- FDA approved plant derived Pharmaceuticals

Drug	Plant Source	Application	Approval Year
Apomorphine	Papaver somniferum	Parkinson's disease	2004
Artemisinin	Artemisia annua (sweet wormwood)	Antimalarial	2020
Atropine	Atropa belladonna (deadly nightshade)	Anticholinergic	1950s
Capsaicin	Capsicum spp. (chili peppers)	Analgesic	1991
Colchicine	Colchicum autumnale (autumn crocus)	Anti-gout	1961
Digoxin	Digitalis lanata (foxglove)	Heart failure	1954
Galantamine	Galanthus spp. (snowdrop)	Alzheimer's disease	2001
Morphine	Papaver somniferum (opium poppy)	Analgesic	1941
Paclitaxel	Taxus brevifolia (Pacific yew)	Anticancer	1992
Quinidine	Cinchona spp. (cinchona)	Antiarrhythmic	1920s
Quinine	Cinchona spp. (cinchona)	Antimalarial	1940s
Reserpine	Rauwolfia serpentina (Indian snakeroot)	Antihypertensive	1954
Vincristine	Catharanthus roseus (Madagascar periwinkle)	Anticancer	1963

9. FUTURE PROSPECTS

The future of phytopharmaceuticals is promising, with trends such as personalized medicine, optimized formulations, biotechnology, integration with conventional medicine, digital tools and data analytics, sustainability, regulatory acceptance, consumer education,

combating antimicrobial resistance, and global collaboration shaping their development. These trends could improve the efficacy, safety, and availability of phytopharmaceuticals, making them a more viable option for healthcare providers and consumers. The future of phytopharmaceuticals is also driven by changing consumer preferences, technological advancements, and the demand for safe healthcare solutions. As research continues to uncover the many health benefits of plant-based compounds, these natural remedies are likely to play an increasingly important role in modern medicine. However, the phytopharmaceutical industry faces several challenges moving forward:

- The need for more clinical trials to demonstrate the safety and efficacy of phytopharmaceuticals.
- The need to develop standardized manufacturing processes to ensure the quality and consistency of phytopharmaceutical products.
- The need to overcome regulatory hurdles in order to market phytopharmaceutical products in different countries.

Despite these challenges, the future of phytopharmaceuticals is bright. As the demand for natural and herbal products continues to grow, the phytopharmaceutical industry is well-positioned to meet this demand.

CONCLUSION

Phytopharmaceuticals are plant-derived products that have been used for centuries to treat a variety of conditions. They are gaining increasing popularity as a natural alternative to conventional medicine. However, the safety and efficacy of phytopharmaceuticals are still being investigated. Some phytopharmaceuticals have shown effectiveness in clinical trials, while others have not. There is also concern about the potential toxicity of some phytopharmaceuticals. The quality of phytopharmaceuticals can vary significantly. Some are standardized to ensure consistent amounts of active ingredients, while others are not. The regulatory status of phytopharmaceuticals also varies from country to country. In some nations, they are regulated as dietary supplements, while in others, they are regulated as drugs.

Despite these challenges, phytopharmaceuticals have the potential to be safe and effective treatments for a variety of conditions. More research is needed to confirm their safety and efficacy, and to develop standardized manufacturing processes and quality control measures.

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مراجعة لأساليب السلامة والجودة والتنظيم والتسليم الخاصة بالمستحضرات الصيدلانية النباتية

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ملخص

المستحضرات النباتية الدوائية هي مركبات مشتقة من النباتات تتمتع بمجموعة واسعة من الفوائد الصحية المحتملة. خصائصها الفريدة وتطبيقاتها المتعددة يجعلها مرشحة واعدة لعلاج العديد من الأمراض. المستحضرات النباتية الدوائية تحتوي على مجموعة واسعة من المركبات الحيوية النشطة، بما في ذلك القلويدات والتربينات والفلافونيدات. هذه المركبات لها مجموعة متنوعة من الأنشطة البيولوجية، بما في ذلك الأنشطة المضادة للأكسدة والمضادة للالتهابات والمضادة للميكروبات. في السنوات الأخيرة، زاد الاهتمام بالمستحضرات النباتية الدوائية لعلاج مجموعة متنوعة من الحالات، بما في ذلك السرطان وأمراض القلب والأوعية الدموية وداء السكري. ومع ذلك، هناك حاجة إلى المزيد من الأبحاث لفهم تمامًا فعالية وسلامة هذه المركبات. لضمان الجودة والسلامة للمستحضرات النباتية الدوائية، تم تطوير إجراءات إدارة الجودة استنادًا إلى مبادئ منظمة الصحة العالمية وممارسات الزراعة والتجميع الجيدة. فهم هذه اللوائح أمر ضروري لضمان تطوير المنتجات الدوائية النباتية فعالية وتصنيعها وتوزيعها. بينما أظهرت المستحضرات النباتية الدوائية وعدًا في الأبحاث المختبرية، هناك تحديات في ترجمة فعاليتها إلى تطبيقات سريرية فعالة، خاصة فيما يتعلق بالتسليم. هناك حاجة إلى مقاربات مبتكرة، مثل طرق التسليم المستهدفة واستراتيجيات الجسيمات النانوية، للتغلب على هذه التحديات. تقدم هذه المراجعة نظرة شاملة على التحديات والفرص في مجال المستحضرات النباتية الدوائية.

الكلمات الدالة: المستحضرات النباتية الدوائية، الجودة، التنظيمات، التحديات، المستحضرات النباتية الدوائية المعتمدة من إدارة الغذاء والدواء الأمريكية .(FDA).

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LC-MS Analysis of Secondary Metabolites of *Asphodelus aestivus* Brot. (Asphodelaceae) grown wild in Jordan

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ABSTRACT

The phytochemical composition of *Asphodelus aestivus* Brot., a plant with therapeutic properties in traditional medicine, remains largely unexplored, particularly in the specific environmental conditions of Jordan. This study utilized advanced LC-MS techniques to comprehensively analyze the secondary metabolites of a plant species endemic to Jordan. The development of the LC-MS method involved optimizing parameters such as solvent composition, gradient elution, and ionization techniques to achieve comprehensive metabolite profiling. The method was validated to ensure accurate, precise, sensitive, and specific identification and quantification of the compounds. Our analysis identified seven distinct compounds, including both familiar molecules and more complex anthrones and glycosides. This finding emphasizes the wide range of chemical compounds found in the plant and highlights the distinct chemical variations influenced by regional environmental factors. These findings contribute to our understanding of *Asphodelus aestivus* Brot. and highlight the potential therapeutic uses of its distinct phytochemical composition. This research makes a significant contribution to the field of plant-based natural products by combining modern analytical methods with traditional medicinal knowledge to investigate the complex phytochemical composition of *Asphodelus aestivus*.

Keywords: *Asphodelus aestivus* Brot; Phytochemical profile; Jordanian cultivation; LC-MS analysis; Secondary metabolites; Traditional medicinal plant.

1- INTRODUCTION

Asphodelus aestivus Brot., a member of the Asphodelaceae family [1], plays a vital role in both the plant kingdom and traditional medicine. Asphodelus aestivus Brot. has been traditionally used in different cultures for its significant anti-inflammatory, antioxidant, and antimicrobial properties [2-5]. The therapeutic applications of this plant highlight its potential contribution to the development of pharmacological products derived from natural sources. Previous studies have predominantly examined Asphodelus aestivus Brot. utilizing conventional extraction techniques, which have often restricted the investigation to a specific set of metabolites [6]. Moreover, many studies have focused on samples cultivated in particular geographic areas, as illustrated in Figure 1 based on [7, 8], compared with our study in Jordan.

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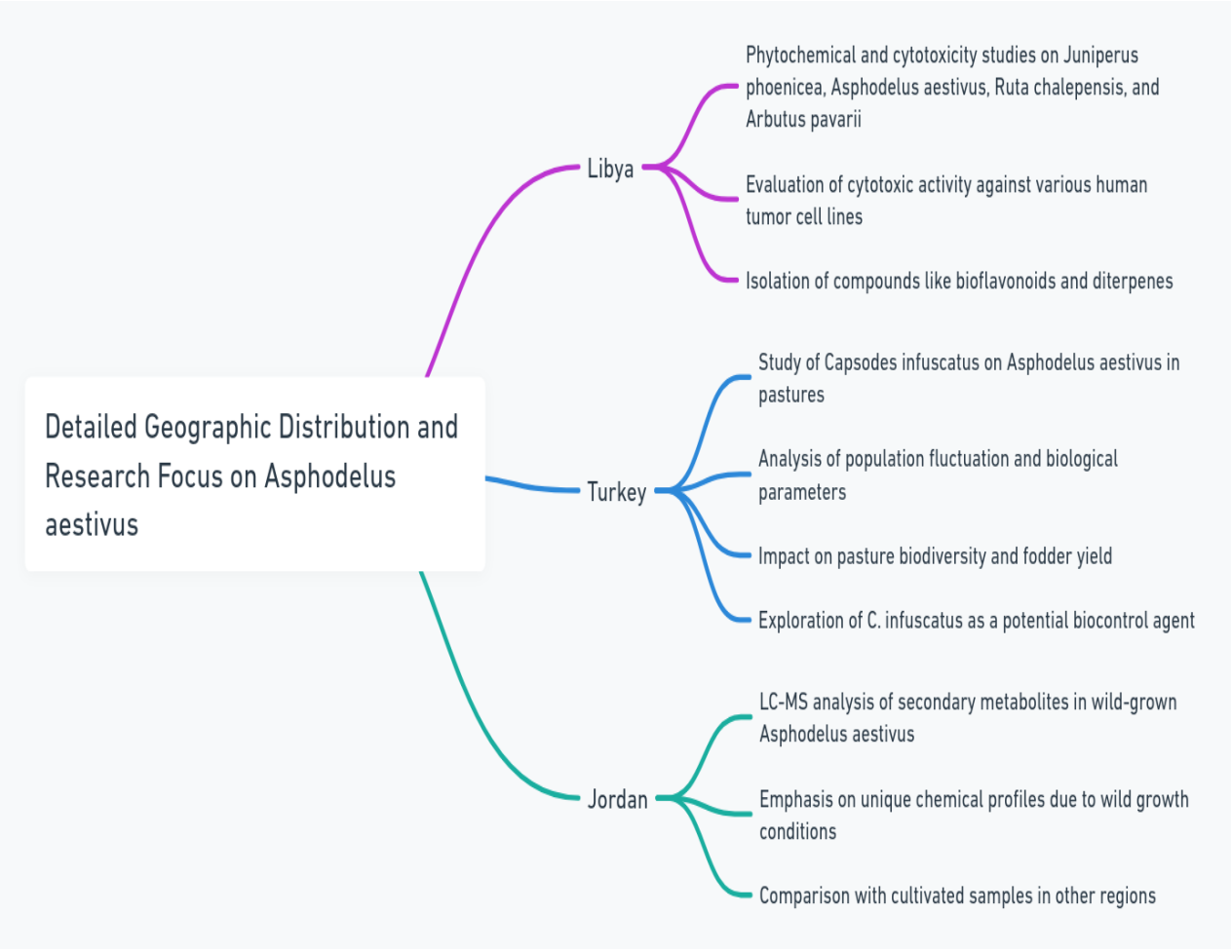

Figure 1: Geographic Distribution and Research Focus of Asphodelus aestivus. The diagram depicts the research approaches employed in Libya, Turkey, and Jordan, particularly focusing on the current utilization of LC-MS analysis for studying wild-grown specimens in Jordan.

This approach neglects the significant impact of environmental factors on the phytochemical composition of the plant. The ecological conditions in Jordan, specifically its diverse environment, have the potential to greatly influence the secondary metabolite profile of plants. However, this aspect has not been extensively studied in the existing literature. Our study suggests the utilization of advanced LC-MS techniques [9, 10], taking into account these factors, as illustrated in Figure 2.

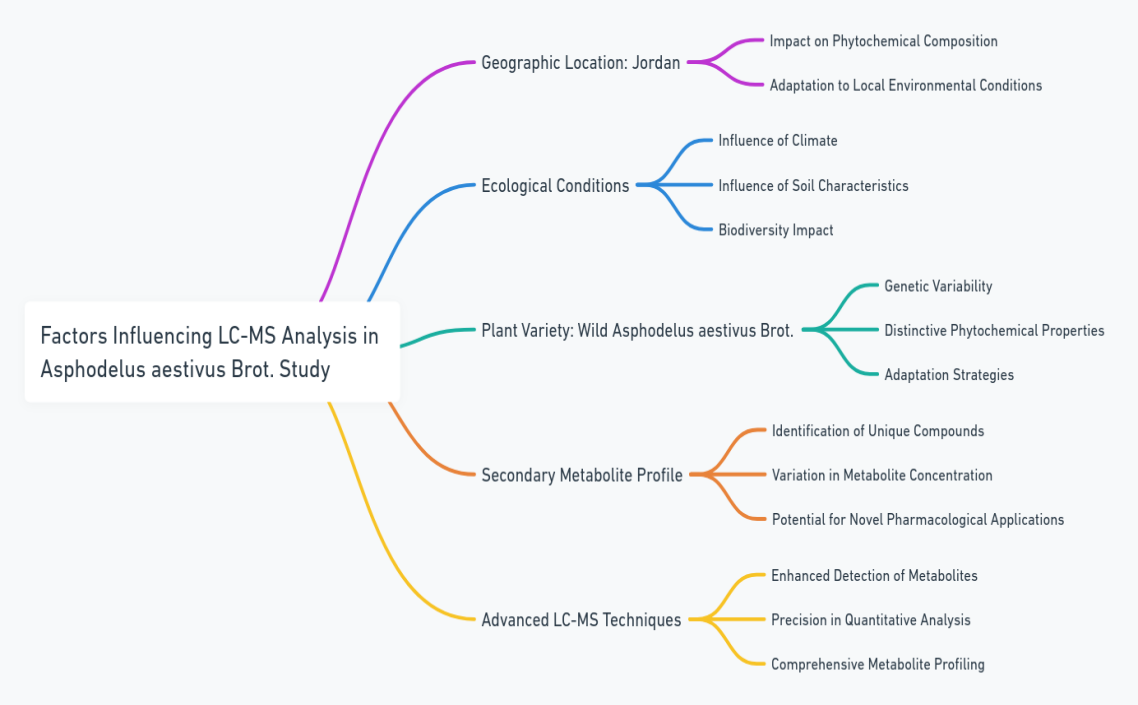

Figure 2 Factors in LC-MS Analysis of Asphodelus aestivus Brot.: This mind map highlights the key elements that impact LC-MS studies of Asphodelus aestivus Brot., focusing on geographic, ecological, and plant-specific factors. Additionally, it emphasizes the importance of advanced analytical techniques in identifying distinct pharmacological compounds found in the native Asphodelus aestivus Brot. of Jordan.

This approach allows for a thorough analysis of the secondary metabolites in *Asphodelus aestivus* Brot., specifically focusing on wild varieties cultivated in Jordan. The objective is to offer a comprehensive analysis of the phytochemical composition specifically suited to the unique ecological context. This perspective is expected to reveal a wider range of secondary metabolites, potentially uncovering unique compounds specific to the Jordanian environment. The findings present potential for pharmacological applications by utilizing the diverse range of phytochemicals in the region. The main objective of this study is to analyze and categorize the secondary metabolites of *Asphodelus aestivus* Brot. discovered in Jordan using advanced LC-MS techniques. Our objectives are to enhance the existing literature in the fields of phytochemistry and pharmacology, with a particular focus on plant-based natural products, and to bridge the gap between traditional herbal knowledge and modern scientific investigation, thereby making a significant contribution to ongoing research in natural product discovery.

2- MATERIALS AND METHODS

2.1- Materials

Experimental Standards: Chrysophenol, aloe-emodin, and frangulin B, sourced from Sigma-Aldrich. Chemicals, Solvents, and Reagents: All of ACS analytical grade, procured from Sharluo, Spain.

2.2- Plant Collection and Classification

Location and Time of Collection: Rhizomes of Asphodelus aestivus collected from Rujm Al-Shoof area, 15 km northeast of Amman, in March 2019.

Taxonomic Verification: Conducted by Dr. Mohammad Gharaibeh, Faculty of Agriculture, Jordan University of Science and Technology. Comparison with voucher specimens at the faculty's herbarium.

Voucher Specimen: Deposited in the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology (ID No.: Phar 09-4).

2.3- General Experimental Procedures:

Column Chromatography (CC): Conducted using silica gel (63-200 µm, Merck, Germany).

Thin Layer Chromatography (TLC): Utilized pre-coated Kiese gel 60 F254 plates (0.2 mm, Merck, Germany). Detection through Ce(SO4)2 / H2SO4 treatment.

Preparative TLC: Employed Silica gel GF254 (20×20 cm, 1 mm thickness).

2.4- Extraction and Purification:

Initial Processing: Dried rhizomes ground and extracted using 70% methanol, followed by 50% methanol.

Defatting and Concentration: Crude extracts defatted with petroleum ether; solvent removal under reduced pressure at 60°C.

Yield and Sub-fractionation: Yield of 15.4% (185g); column chromatography for sub-fractionation into 7 fractions (F1-F7) based on TLC behavior as shown in (Table 1) and illustrated in Figure 3.

Table 1 Summary of Extraction and Identification of Compounds

Fraction	Mass (mg)	Compound	Mass of Compound (mg)	Yield (%)	Method of Isolation	Method of Confirmation
1	15	Chrysophanol (Compound 1)	6	40	n-hexane/EtOAc elution	co-TLC, LC-MS
1	15	Aloe-emodin (Compound 2)	2	13.3	n-hexane/EtOAc elution	co-TLC, LC-MS
2	35	Chlorogenic Acid (Compound 3)	2	8.7	Preparative TLC (CHCl3: MeOH, 90:10, v/v)	co-TLC, LC-MS
3	23	Frangulin B (Compound 4)	1.2	5.2	Preparative TLC (CHCl3: MeOH, 90:10, v/v)	co-TLC, LC-MS
4	18	10-(Chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone (Compound 5)	3.2	17.8	Preparative TLC (CHCl3: MeOH, 95:5, v/v)	LC-MS
5 & 6 (Pooled)	38	Ramosin (Subfraction 1)	7	18.4	Sephadex LH-20 Column (Methanol)	LC-MS
5 & 6 (Pooled)	38	Aspheloside B (Subfraction 2)	9.2	24.2	Sephadex LH-20 Column (Methanol)	LC-MS

Note: Isolation methods such as n-hexane/EtOAc elution and Sephadex LH-20 column chromatography were used for fraction separation. Compounds were confirmed using co-thin layer chromatography (co-TLC) with standards and liquid chromatography-mass spectrometry (LC-MS) analysis.

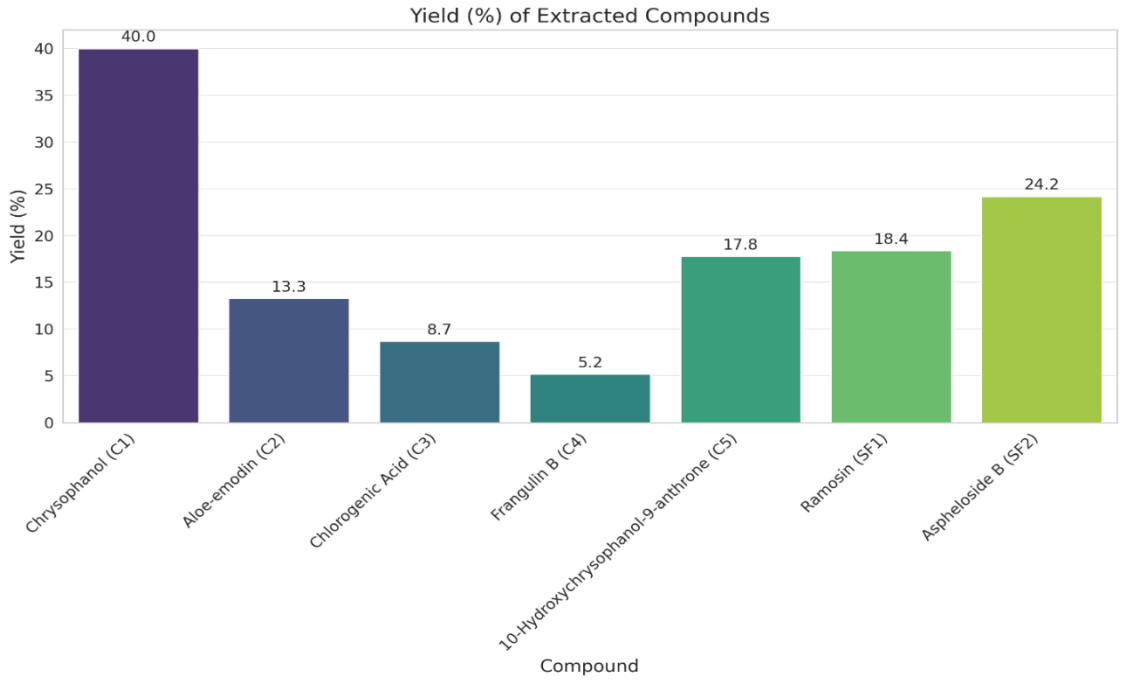

Figure 3 Figure investigates the comparative yield percentages of extracted compounds (C1 to SF2) across different fractions. The bar chart illustrates the efficiency of different isolation methods and their impact on yield outcomes.

2.5- Sample Preparation for LC-MS:

Solubilization: The phytochemical constituents present in the final extract were dissolved to obtain a uniform concentration of 10.0 mg/100 ml in anhydrous methanol. The solvent was chosen based on its ideal compatibility with the organic compounds and minimal impact on the efficiency of ionization in mass spectrometry.

Dilution Optimization: The methanolic solution was diluted deliberately to adjust the analyte's concentrations to match the LC-MS detector's dynamic range. This was done to improve the signal-to-noise ratio and ensure reliable analytical performance.

Microfiltration: A 0.45 µm PTFE syringe filter was used to achieve sterility and eliminate sub-micron particulate contaminants. This step is crucial for preserving the integrity of the analytical column and improving the accuracy of the mass spectrometric analysis.

2.6- LC-MS Analysis:

Instrumentation: Phenomenex Gemini C18 column (250 mm x 4.6 mm i.d.; 5 µm particle size).

Conditions: Negative ion mode, full scan spectra (m/z 50-900), and MS/MS fragmentation on selected ions. Nebulization and drying with high-purity nitrogen at specified temperatures and flow rates.

Data Analysis: Utilizing Analyst 1,6.3 software (Germany-Darmstadt).

The mobile phase

Solution A: Water mixed with 0.005M ammonium acetate and 0.1% acetic acid. Solution B: Acetonitrile mixed with 0.005M ammonium acetate and 0.1% acetic acid. The mobile phase for the LC-MS analysis consisted of a combination of Solutions A and B. The gradient elution was performed at a constant flow rate of 1.0 µL/min as shown in (Table 2).

Table 2: Gradient Elution Steps

Step	Total Time(min)	Flow Rate(µl/min)	A (%)	B (%)
0	0.00	1000	90.0	10.0
1	5.00	1000	90.0	10.0
2	15.00	1000	10.0	90.0
3	20.00	1000	10.0	90.0
4	25.00	1000	90.0	10.0
5	30.00	1000	90.0	10.0

Note: The table above provides a detailed overview of the gradient elution protocol used in the LC-MS analysis. The sequence of steps outlines the changes in the concentration of Solutions A and B over time, essential for the separation and identification of compounds.

3- RESULTS AND DISCUSSION

We have successfully identified seven distinct compounds in the extract of *Asphodelus aestivus* using LC-MS analysis. The compounds mentioned in Table 3 encompass various molecules, including Chrysophanol, Aloe-emodin, Chlorogenic acid, Frangulin B, as well as complex anthrones and glycosides such as 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone, Aspheloside B, and Ramosin.

Table 3: Mass Spectrometry Results for Compounds Extracted from Asphodelus aestivus

Compound	Formula	MS Peak	Molecular Ion Peak	Major Fragments	Retention Time, (min)
Chrysophanol (1)	C15H10O4	m/z 252.7	m/z 253 (C15H10O4+)	m/z 253 (C15H9O4.), m/z 239 (C14H7O4.)	17.85
Aloe-emodin (2)	C15H10O5	m/z 269	m/z 270 (C15H10O5+)	m/z 253 (C15H9O4.), m/z 239 (C14H7O4.)	15.15
Chlorogenic acid (3)	C16H18O9	m/z 354.7	m/z 355 (C16H19O9+)	m/z 191 (C7H11O6.), m/z 163 (C9H7O3.)	12.84
Frangulin B (4)	C20H18O9	m/z 401	m/z 402 (C20H19O9+)	m/z 253 (C15H9O4.), m/z 149 (C5H9O5.)	10.35
10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone (5)	C30H20O8	m/z 505	m/z 506 (C30H21O8+)	m/z 238.06 (C15H10O32.), m/z 253.05 (C15H9O4.)	20.45
Aspheloside B (6)	C35H27O11	m/z 639	m/z 640 (C35H28O11+)	m/z 507 (C30H19O8.)	16.27
Ramosin (7)	C36H31O12	m/z 671	m/z 672 (C36H32O12+)	m/z 492, m/z 255	14.7

Note: This table summarizes the mass spectrometry results for seven key compounds identified in Asphodelus aestivus. The data includes the molecular formula, mass-to-charge ratio (MS Peak), molecular ion peak, major fragment ions, and retention times, providing insights into the molecular structures and fragmentation patterns of each compound.

DISCUSSION

Analysis of Molecular Ion Peaks:

The molecular ion peaks played a crucial role in the identification of the compounds present in *Asphodelus aestivus*. Chrysophanol (1) and Aloe-emodin (2) exhibited molecular ion peaks at m/z 253 and m/z 270, respectively, confirming their respective structures. Chlorogenic acid (3) and Frangulin B (4) were analyzed using mass spectrometry, providing significant information about their molecular structures. Chlorogenic acid exhibited a peak at m/z 355, while Frangulin B showed a peak at m/z 402. These findings offered valuable insights into the configurations of these compounds, as illustrated in Figure 4.

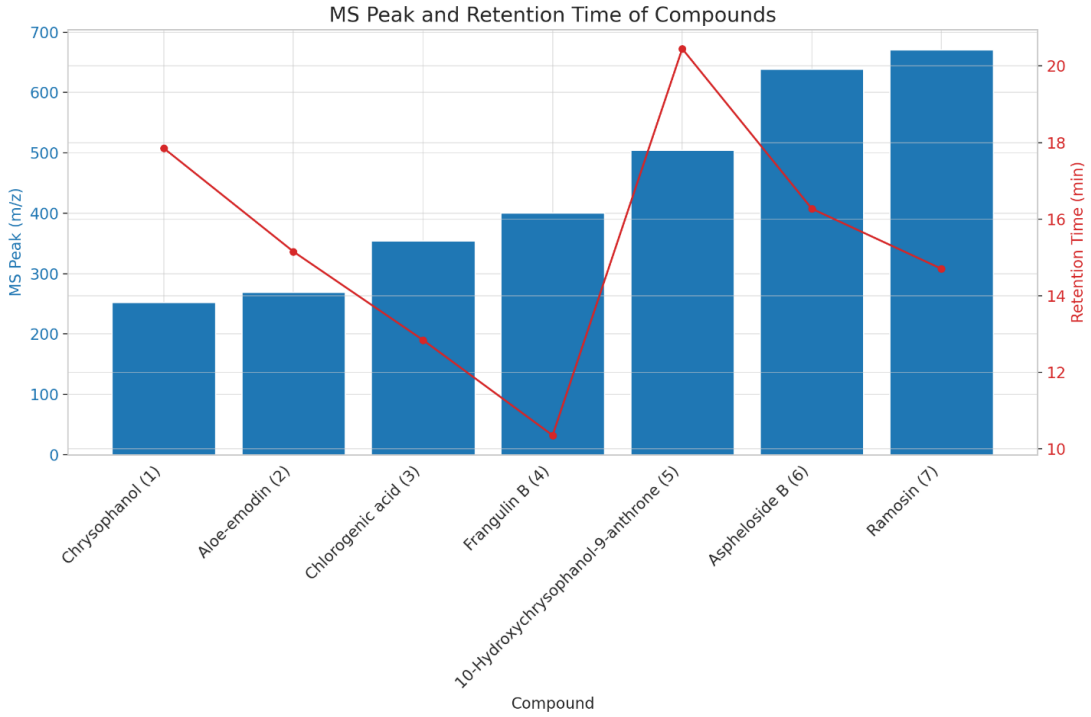

Figure 4 Correlation between Mass Spectrometry Peaks (m/z) and Retention Times (min) for Specific Compounds. The graph illustrates a dual-axis representation, where blue bars represent MS Peaks and a red line represents Retention Times. This format compares mass-to-charge ratios and chromatographic retention characteristics of compounds, specifically Chrysophanol (1) to Ramosin (7).

Insights from Advanced Compound Analysis:

The identification of compounds in our study relied heavily on the presence of distinct molecular ion peaks. Compound 5, known as 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone, exhibited a prominent peak at m/z 506. Compound 6, also referred to as Aspheloside B, displayed a significant [M+H]+ ion at m/z 640, with the molecular formula C30H20O8. The peak at m/z 640 observed for Aspheloside B is consistent with prior studies [11-16] and played a crucial role in confirming its molecular composition. The cleavage of the C10–C6′ bond in Aspheloside B resulted in significant fragments at m/z 238.06 and 253.05, providing valuable insights into its intricate molecular structure. These results highlight the complex molecular nature of Aspheloside B and underscore the effectiveness of mass spectrometry in revealing detailed compound characteristics.

Furthermore, Ramosin (7) exhibited a notable peak at

m/z 672, offering valuable insights into its potential biological functionalities. The mass spectrometry analysis of Ramosin revealed a significant peak at m/z 655 (C36H32O12+), indicative of a hydroxyl (OH) group removal from the carbon atom at position 10'. Additional fragmentation produced a notable ion at m/z 492, suggesting phenolic glycosides with included glucose units [17, 18]. The presence of substantial mass units at m/z 255 suggests the likely presence of a chrysophenol (1) component in the compound [17-19].

Overall, the molecular ion peaks and fragmentation patterns observed in our analysis facilitated the identification of these compounds, providing insights into their molecular structures and potential bioactivities.

Interpretation of Fragmentation Patterns:

Chrysophanol (1) and Aloe-emodin (2) exhibited fragmentation patterns consistent with the absence of hydroxyl and carbonyl functional groups. Frangulin B (4) displayed fragmentation, resulting in the production of chrysophanol and 3-(D-Apio-β-D-furanosyloxy) compounds, indicating involvement in an intricate biosynthetic pathway. Moreover, Aspheloside B (6) exhibited a notable reduction in mass units, specifically from m/z 253 to 162, suggesting the elimination of a phenolic group. This fragmentation pattern is a distinctive characteristic of this compound. The fragmentation pattern of Ramosin (7) elucidates its intricate structure and potential biosynthetic pathways by identifying specific ions formed and their mass-to-charge ratios.

Pharmacological Implications:

The discovery of distinct compounds in *Asphodelus aestivus*, particularly those exclusive to Jordan, presents promising opportunities for pharmacological use. The compound 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone (5) exhibits an intricate structure with potential interactions with distinct biological pathways. Due to its distinctive molecular structure, *Asphodelus aestivus* shows potential as a candidate for anti-inflammatory or antioxidant properties, aligning with its traditional uses. Further exploration of these potential activities through detailed bioassays and in-vivo studies is recommended. These studies may provide valuable insights into the therapeutic mechanisms of these compounds, potentially leading to the development of novel pharmacological agents.

Environmental Influences on Phytochemical Profile:

Our research indicates that Jordan's unique environmental factors, including soil composition, climate, and local biodiversity, significantly influence the phytochemical composition of *Asphodelus aestivus*. The observed differences in compound profiles between Jordanian samples and those from other regions suggest that the distinctive environmental conditions in Jordan may stimulate the production of specific secondary metabolites, including recently discovered compounds. This aligns with the concept of 'phytochemical plasticity'. Further research is necessary to fully understand the precise impact of environmental factors on the biosynthesis of these compounds. This knowledge could have profound implications for developing sustainable harvesting and cultivation practices.

CONCLUSION

The therapeutic uses of *Asphodelus aestivus* Brot. have been extensively documented. However, the phytochemical composition of this plant, specifically when cultivated in Jordan's unique environment, has not yet been studied. Using advanced liquid chromatography-mass spectrometry (LC-MS) techniques, we have successfully identified seven distinct compounds. This discovery sheds light on the diverse chemical composition of the plant and the intricate pathways involved in its biosynthesis. Our research expands the current knowledge of *Asphodelus aestivus* Brot. and highlights its unexplored possibilities in region-specific investigations. This study

combines modern analytical methods with traditional knowledge to pave the way for future investigations into plant-based natural products. It also provides opportunities for potential therapeutic advancements.

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Conflicts of interest

The authors have stated that there is no conflict of interest associated with the publication.

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تحليل الكروماتوغرافيا السائلة المزدوجة مع الطيف الكتلي للمركبات الاستقلابية الثانوية لنبات أسفوديلوس أيستيفوس بروت (فصيلة الأسفوديلية) النامي بريًا في الأردن

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ملخص

أُظهرت الدراسات أن نبات أسفوديلوس أيستيفوس بروت يُستخدم على نطاق واسع في الطب التقليدي لخصائصه العلاجية. ومع ذلك، لا يزال هناك نقص في المعرفة حول المكونات الكيميائية النباتية لهذا النبات، خاصة عند نموه في الأردن. في هذه الدراسة، تم استخدام تقنيات الكروماتوغرافيا السائلة المزدوجة مع الطيف الكتلي المتقدمة لدراسة المركبات الاستقلابية الثانوية لهذا النوع من النباتات النامية بريًا في الأردن. كشفت نتائجنا عن وجود سبعة مركبات مختلفة، تشمل مركبات معروفة وأنثرونات وجليكوسيدات معقدة، مما يُبرز التنوع الكيميائي للنبات. تُسهم هذه النتائج في فهمنا لأسفوديلوس أيستيفوس بروت وتُظهر التباين الكيميائي بحسب المناطق، بالإضافة إلى التطبيقات العلاجية المحتملة. وتعتبر هذه الأبحاث إضافة قيمة للمنتجات الطبيعية النباتية من خلال دمج الطرق التحليلية المعاصرة مع المعرفة التقليدية.

الكلمات الدالة: أسفوديلوس أيستيفوس بروت؛ المكونات الكيميائية النباتية؛ زراعة الأردن؛ تحليل كروماتوغرافيا مزدوجة؛ مركبات استقلابية ثانوية؛ نبات طبي تقليدي.

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Advancements and Challenges in Aptamer-Based Therapeutics and Diagnostics Across Diverse Medical Domains: A Comprehensive Review

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ABSTRACT

Aptamers, which are single-stranded DNA or RNA molecules, are increasingly recognized as important tools in diagnostics and therapeutics across various medical disciplines such as oncology, respiratory diseases, and neurological disorders. This review provides a comprehensive evaluation of the recent progress and obstacles encountered in the field of aptamer-based applications. Aptamers have shown promise in oncology for early cancer detection and targeted drug delivery, effectively reducing off-target effects. They also hold potential for significantly impacting the management of respiratory conditions such as asthma and Chronic Obstructive Pulmonary Disease (COPD) by selectively targeting cytokines and regulating the inflammatory response. In the realm of neurological disorders, aptamers offer novel methods by influencing the gut-brain axis and proposing potential approaches for early detection and specific therapy. Despite these notable benefits, persistent challenges remain in areas such as molecular stability, delivery mechanisms, and economic viability. This review offers a comprehensive overview of aptamer-based diagnostics and therapeutics while exploring potential avenues for future research.

Keywords: Aptamer Therapeutics, Diagnostic Aptamers, Immune Modulation, Mind-Gut Axis, Tumor-specific Markers.

INTRODUCTION

Aptamers have revolutionized molecular medicine, significantly impacting our approach to various health conditions. Aptamers show potential in disease diagnostics due to their precise target-binding capabilities [1], targeted therapies [2], and drug delivery [3]. This review explores a wide range of topics related to cancer [4], autoimmune diseases [5], metabolic dysfunctions [6], and mental well-being [7].

Aptamers have emerged as novel tools against cancer, a prevalent human health condition. The potential of precision diagnosis and targeted therapeutics [8-10] could offer promising prospects for enhancing the efficacy of cancer treatments. In autoimmune disorders, aptamers hold promise for facilitating more precise approaches to diagnosis and treatment.

Aptamers present a promising and innovative strategy for managing digestive disorders and metabolic dysfunctions. Their potential application in conditions such as malabsorption could lead to significant advancements in treatment methods [11], inflammatory bowel diseases [12], diabetes [13], and obesity [14].

Furthermore, aptamers play a crucial role in combating infectious diseases [15]. Significant progress has been made in the field of early detection and targeted treatments. Aptamers have also shown potential as therapeutic agents for promoting bone health, expanding their applications beyond traditional biomedical fields [16], and addressing psychological disorders [17].

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This review highlights the various applications of aptamers, emphasizing their significant potential for advancements in health and medicine. It also anticipates a promising future impact by these molecules, as illustrated in Figure 1.

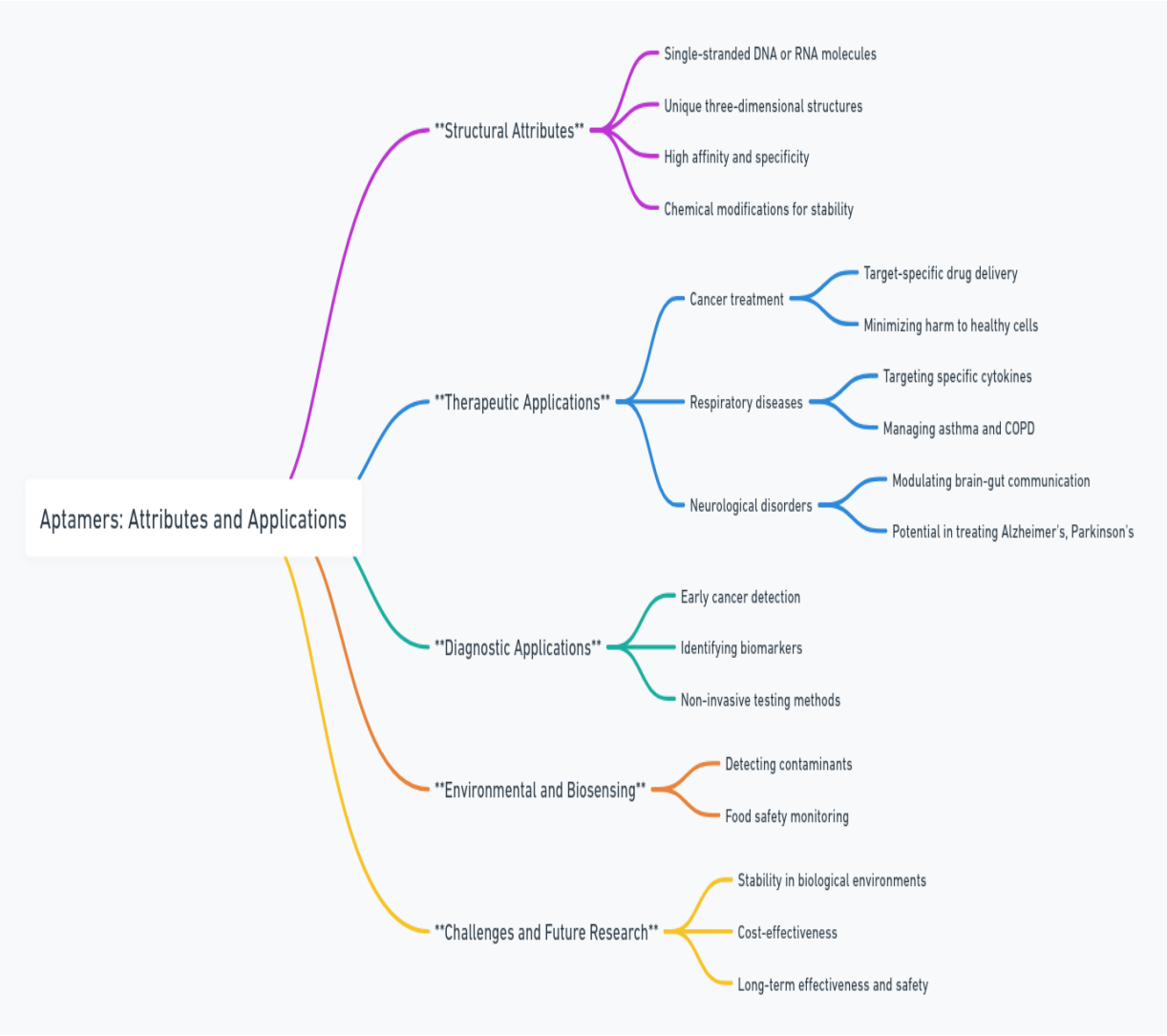

Figure 1 Aptamers: Structural Complexity, Therapeutic Versatility, and Developmental Challenges. This schematic illustrates the structural properties of aptamers, their applications in therapy and diagnosis, and the challenges encountered in their development and clinical use.

History:

The narrative of aptamers begins in the late 20th century, during an exciting era of discovery in the field of molecular biology [18]. Aptamers, referred to as "chemical antibodies," originate from the Latin term "aptus," meaning "to fit," and the Greek term "meros," meaning "part." Their development reflects these etymological origins [19].

The concept of aptamers emerged in the laboratories of two separate research groups, led by Larry Gold and Jack Szostak, during the early 1990s [20]. These scientists embarked on parallel paths to develop molecules that could specifically bind to target molecules, akin to antibodies, but with more versatility [21]. They employed a process known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX), which facilitated the selection of short, single-stranded DNA or RNA molecules with high affinity for specific target molecules [22].

In 1990, Andrew Ellington and Jack Szostak first demonstrated the in vitro selection of RNA ligands, coining the term "aptamers". Concurrently, Larry Gold and Craig Tuerk demonstrated SELEX with RNA binding to bacteriophage T4 DNA polymerase [23]. The advent of the SELEX process marked a pivotal milestone, enabling the creation of aptamers targeting diverse entities.

The period following SELEX witnessed a transition towards enhancing the stability and functionality of aptamers through optimization. During this time, chemically modified aptamers emerged, increasing resistance to nuclease degradation and improving binding affinities [24].

Therapeutic aptamers emerged in the early 21st century. The FDA approved Macugen (pegaptanib) in 2004 as the first aptamer-based drug for treating neovascular age-related macular degeneration (AMD). This significant milestone confirmed the therapeutic capabilities of aptamers and spurred further research and development efforts.

Aptamers have become valuable tools in various domains, including therapeutics, diagnostics, and environmental sensing. The development and utilization of aptamers have undergone significant progress, leading to their widespread application in precision medicine and laying a solid groundwork for future innovations.

Structural and Functional Characterization of Aptamers

Aptamers are a crucial and adaptable component of contemporary molecular medicine. Known as "chemical antibodies," these short single-stranded DNA or RNA molecules possess remarkable recognition and binding capabilities, displaying high affinity and specificity towards diverse target molecules [25], ranging from small organic compounds and metal ions to large proteins and entire cells. Unlike antibodies, which rely on their distinctive three-dimensional structures for binding, aptamers exhibit unparalleled versatility due to their ability to fold into precise shapes that match their targets [26]. This flexibility allows them to adapt their structure precisely to specific targets, thereby demonstrating strong binding capabilities.

Aptamers have significantly transformed the field of molecular recognition and interaction, serving as valuable tools in biomedical applications. The chemical synthesis of these compounds ensures consistent quality and performance. Additionally, their thermal stability and non-immunogenic nature facilitate convenient modification or labeling without compromising their binding capabilities [27]. Aptamers possess the unique ability to bind targets that are difficult for antibodies to recognize, either due to their toxicity or lack of immunogenic properties.

Advances in Aptamer Selection

The SELEX process (Systematic Evolution of Ligands by Exponential Enrichment) is a highly effective method for identifying and optimizing aptamers, demonstrating exceptional specificity. The process begins with a large reservoir containing a diverse range of oligonucleotide sequences, estimated to be up to 10^15 in number [28].

The target molecule selectively allows the survival of molecules that can bind to it effectively, mimicking natural selection. This selection is facilitated through the partitioning and amplification of higher affinity oligonucleotides via PCR in successive rounds of selection. SELEX is versatile, functioning effectively in both physiological and harsh environments [29], and is highly valuable for applications in diagnostics and therapeutics.

Moreover, the integration of Next-Generation Sequencing (NGS) with SELEX techniques has had a transformative impact. This integration has not only facilitated the consolidation of millions of DNA sequences but has also enhanced scientists' ability to identify rare yet highly specific aptamers with improved precision and efficiency [30]. NGS with SELEX provides researchers opportunities to investigate sequence diversity and enhance the selection process from existing aptamer libraries.

Advancements and Challenges in Enhancing Aptamer Stability and Specificity

Aptamers possess several advantages, yet their stability and specificity require enhancement for increased practicality. Scientists have addressed the issue of nucleases degrading aptamers in biological environments by implementing innovative approaches. These include incorporating chemical modifications such as 2'-fluoro, 2'-amino, and 2'-O-methyl substitutions into the aptamer's sequence structure. Additionally, protective carriers like nanoparticles and liposomes have been utilized to enhance resistance against nucleases [31]. Advances in specificity have been achieved through refined Systematic Evolution of Ligands by Exponential Enrichment (SELEX) protocols and advanced bioinformatics tools. These developments have enabled a more precise selection process for non-targets and a deeper understanding of the interaction between aptamers and targets.

Aptamers and Gastrointestinal Health

As research on the gastrointestinal (GI) tract's impact on health and disease expands, the therapeutic and diagnostic applications of aptamers also show promise. The complex nature of this system makes it vulnerable to various diseases, such as inflammatory bowel diseases and gastric cancers, providing a favorable opportunity for aptamer intervention. Aptamers have demonstrated diagnostic potential for colorectal cancer, a prevalent and highly dangerous gastrointestinal malignancy. They achieve this by identifying and binding to proteins or other molecular markers that are abnormally expressed in the presence of the disease, enabling early detection and facilitating prompt implementation of preventive or minimization treatments.

Aptamers have been identified as a promising tool for targeted treatments in the fight against gastrointestinal (GI) diseases, as indicated in Table 1. They can be engineered to specifically target and inhibit pro-inflammatory cytokines, which play a central role in the development of inflammatory bowel disease (IBD) [32]. This strategy offers an advanced approach with fewer side effects compared to current therapies. Additionally, there is promise in using aptamers to manipulate gut microbiota for therapeutic benefit [33-35], a concept gaining recognition due to its significant role in health and disease.

Aptamers and Oral Health

The oral cavity is a multifaceted ecosystem, hosting a wide array of microorganisms and tissues. It also serves as a pathway to oral diseases, such as periodontal disease and cancer, which can significantly impact overall health and well-being. Aptamers have revolutionized oral healthcare by enabling advancements in diagnostics and treatment monitoring. Salivary aptamers are currently under investigation for their potential application in noninvasive early detection of various conditions, including cancer, a prevalent form of malignancy globally [53, 54]. This method may enable cost-effective saliva tests for early detection of cancer biomarkers, leading to improved treatment outcomes for patients.

Table 1 This table presents a summary of various gastrointestinal conditions, the aptamers used to treat them, their mechanisms of action, and the corresponding scientific references. Each entry describes a specific aptamer's function in disease management, as reported in the literature cited.

Gastrointestinal Condition	Aptamer Types	Mechanism of Action	References
Inflammatory Bowel Disease (IBD)	TNF-α aptamer	Binds to and neutralizes TNF-α, reducing inflammation	[32]
Celiac Disease	Gliadin aptamer	Binds to gliadin, blocking its interaction with the intestinal epithelium	[36]
Gastritis	Leukotriene B4 aptamer	Binds to and inhibits leukotriene B4, reducing inflammation	[37]
Helicobacter pylori Infection	Urease aptamer	Binds to urease enzyme, inhibiting H. pylori colonization	[38]
Hemorrhoids	VEGF aptamer	Inhibits VEGF, reducing hemorrhoidal bleeding	[39]
Crohn's Disease	IL-6 aptamer	Binds to IL-6, reducing inflammation and disease activity	[40]
Irritable Bowel Syndrome (IBS)	5-HT4 aptamer	Binds to 5-HT4 receptors, modulating gut motility	[41]
Diverticulitis	TNF-α aptamer	Binds to and neutralizes TNF-α, reducing inflammation	[42]
Constipation	L-type Calcium Channel aptamer	Modulates L-type Calcium Channel, improving gut motility	[43]
Hepatic Cirrhosis	TGF-β1 aptamer	Binds to and inhibits TGF-β1, preventing liver fibrosis	[44]
Non-alcoholic Fatty Liver Disease (NAFLD)	FABP4 aptamer	Binds to FABP4, reducing lipid accumulation in the liver	[45]
Gastrointestinal Bleeding	Thrombin aptamer	Binds to and inhibits thrombin, preventing blood clot formation	[46]
Gastroenteritis	Aptamer against bacterial toxins	Neutralizes bacterial toxins, preventing damage to the gut lining	[47]
Gastroesophageal Reflux Disease (GERD)	PPI-aptamer	Inhibits proton pump action, reducing stomach acid production	[48]
Ulcerative Colitis	NF-κB aptamer	Inhibits NF-κB, reducing inflammation and promoting mucosal healing	[49]
Pancreatitis	Trypsin aptamer	Inhibits trypsin, reducing inflammation and pancreatic damage	[50]
Gallstones	Cholesterol aptamer	Binds to cholesterol in bile, preventing gallstone formation	[51]
Fatty Liver Disease	SREBP-1c aptamer	Inhibits SREBP-1c, reducing lipid accumulation in the liver	[52]

Abbreviations: TNF: tumor necrosis factor, VEGF: vascular endothelial growth factor, IL: Interleukin, 5-HT: 5-hydroxytryptamine, TGF: Transforming Growth Factor, FABP4: fatty acid-binding protein 4, PPI: Proton Pump Inhibitors, NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells, SREBP: Sterol Regulatory Element-Binding Protein.

Aptamers provide a novel approach for treating oral diseases, as indicated in Table 2. Periodontal disease, characterized by chronic inflammation and tissue damage, can be effectively addressed by utilizing aptamers. These molecules have the ability to specifically target crucial inflammatory mediators or pathogenic bacteria, thereby impeding the advancement of the disease [55]. Furthermore, aptamers have shown significant promise in addressing oral cancer. Specifically, aptamers engineered to bind and inhibit oncogenic proteins within cancer cells selectively have yielded remarkable outcomes in preclinical investigations.

Moreover, the incorporation of aptamers into dental materials has the potential to significantly enhance restorative dentistry. These aptamers can be integrated into materials to confer antimicrobial capabilities or promote tissue regeneration.

Table 2 This table provides a concise overview of various oral conditions, the aptamers used for their management, their mechanisms of action, and the associated references. Each row specifies an aptamer's role in influencing a particular oral condition, as supported by the referenced studies.

Oral Condition	Aptamer	Mechanism of Action	References
Oral Leukoplakia	EpCAM Aptamer	Reduces cell proliferation, potentially halting disease progression	[56]
Halitosis (Bad Breath)	H2S Aptamer	Detects and quantifies halitosis related H2S	[57]
Oral Mucositis	TLR4 Aptamer	Blocks TLR4, reducing inflammation and ulceration	[58]
Oral Candidiasis	Candida Aptamer	Binds to Candida species, preventing biofilm formation	[59]
Oral Lichen Planus	EpCAM Aptamer	Targeting EpCAM positive cells, reducing proliferation	[60]
Oral Ulcers	VEGF165 Aptamer	Inhibits angiogenesis, promoting wound healing	[61]
Gingivitis	TNF-α Aptamer	Blocks TNF-α, reducing inflammation	[62]
Dental Caries	S. Mutans Aptamer	Binds to and inhibits S. Mutans, a leading cause of dental caries	[63]
Periodontitis	RANKL Aptamer	Inhibits RANKL, reducing bone loss in periodontitis	[55]
Dental Plaque	S. Mutans Aptamer	Inhibits S. Mutans, a leading cause of dental plaque	[64]

Abbreviations: EpCAM: Epithelial Cell Adhesion Molecule, H2S: Hydrogen Sulfide, TLR: Toll-Like Receptor, VEGF: vascular endothelial growth factor, TNF: tumor necrosis factor, RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand

Aptamer-Based Targeted Therapy in Oncology

Aptamers in oncology have the potential to revolutionize cancer treatment through targeted therapy [65]. This is exemplified by their successful application in various types of cancer, as shown in Table 3. The AS1411 aptamer exhibits notable versatility by effectively targeting both colorectal and ovarian cancers [66, 67], showcasing the broad applicability of aptamers. AS1411 inhibits cell proliferation by binding to nucleolin, a protein that is overexpressed in numerous cancer cells. This targeted approach minimizes potential harm to healthy tissues, making it highly valuable in the treatment of aggressive cancers such as colorectal cancer [66], where minimizing collateral damage is crucial. It is also beneficial in the case of ovarian cancer, where early detection is challenging, and precise treatments are essential.

The MUC1 aptamer specifically binds to the MUC1

antigen, which is highly expressed in gastric and pancreatic cancers. The specificity of the aptamer allows for targeted interference with tumor cell growth and metastasis. The focused application of the MUC1 aptamer holds great promise in the context of pancreatic cancer [68], a disease known for its resistance to standard therapies and aggressive progression. The MUC1 aptamer has the potential to improve cancer treatment by selectively targeting cancer cells [68], potentially leading to more effective therapies with fewer side effects, which is a significant development in managing these complex malignancies.

The A10 PSMA aptamer demonstrates the potential of aptamers in precision medicine for prostate cancer, a common malignancy known for its high levels of prostate-specific membrane antigen (PSMA) expression [69]. The aptamer's specific binding to PSMA allows for targeted elimination of cancer cells, presenting a promising treatment approach that may improve effectiveness and minimize the typical side effects associated with prostate cancer therapies.

These diverse applications demonstrate the versatility and precision of aptamers in targeting various cancer types. The effectiveness of aptamers such as AS1411 in treating multiple types of cancers, along with the specific targeting capabilities of aptamers like MUC1 and A10 PSMA, underscores the potential of aptamers to significantly enhance personalized cancer therapy.

Table 3 This table summarizes different cancer types, the respective aptamers used for their treatment, their mechanisms of action, and the corresponding research references. Each entry illustrates how a specific aptamer works against a particular type of cancer, as detailed in the cited research.

Cancer Type	Aptamer	Mechanism of Action	References
Colorectal Cancer	AS1411 Aptamer	Binds nucleolin and inhibits cancer cell proliferation	[66]
Gastric Cancer	MUC1 Aptamer	Targets MUC1 on cancer cells and inhibits proliferation	[68]
Prostate Cancer	A10 PSMA Aptamer	Binds to PSMA, selectively targeting and killing prostate cancer cells	[69]
Ovarian Cancer	AS1411 Aptamer	Binds nucleolin, inhibiting cancer cell proliferation	[67]
Skin Cancer	VEGF Aptamer	Inhibits angiogenesis, hindering tumor growth	[70]
Breast Cancer	Her2 Aptamer	Targets Her2, selectively killing breast cancer cells	[71]
Pancreatic Cancer	MUC1 Aptamer	Binds to MUC1 on pancreatic cancer cells, inhibiting proliferation	[72]
Cervical Cancer	EpCAM Aptamer	Binds to EpCAM on cancer cells, inhibiting proliferation	[73]
Bladder Cancer	EpCAM Aptamer	Targets EpCAM on bladder cancer cells, inhibiting proliferation	[74]
Lung Cancer	AXL Aptamer	Targets AXL receptor, inducing apoptosis in cancer cells	[75]
Liver Cancer	ASGPR Aptamer	Targets ASGPR on liver cancer cells, inhibiting proliferation	[76]

Abbreviations: MUC: Mucin, PSMA: Prostate-Specific Membrane Antigen, VEGF: vascular endothelial growth factor, HER: Human Epidermal Growth Factor Receptor, EpCAM: Epithelial Cell Adhesion Molecule, ASGPR: Asialoglycoprotein Receptor.

Aptamers and Airway Inflammation

Managing the inflammatory nature of respiratory diseases such as asthma and COPD poses significant challenges. Aptamers offer an ideal solution as they minimize side effects associated with conventional treatments, which lack specificity. Aptamers are single-stranded DNA or RNA molecules that possess a distinct ability to recognize and bind to specific targets due to their unique three-dimensional structure [20]. This enables them to bind strongly yet specifically to proteins, small molecules, and even cells without inducing any immune response while simultaneously avoiding off-target effects

due to their size [77].

Tralokinumab, an aptamer designed to target interleukin-13 (IL-13), has demonstrated remarkable potential in clinical trials. It has exhibited substantial efficacy in enhancing lung function and managing symptoms among individuals with asthma [78]. Similarly, TNF alpha, another key player in COPD pathogenesis, is being investigated using aptamers, and preclinical models show promising results as well [79].

Aptamers show significant potential in modulating inflammation related to respiratory diseases like asthma and COPD, offering promising opportunities to enhance existing therapeutic approaches. Aptamers are increasingly recognized as valuable diagnostic tools, offering an efficient and non-invasive approach to detect respiratory illness-associated biomarkers [80, 81].

Emerging Applications of Aptamers in Neurological Disorders

The utilization of aptamers in the field of neurological disorders signifies a notable advancement, providing accurate and focused interventions for a range of conditions as shown in Table 4. The X-Aptamer's application in schizophrenia diagnosis represents a significant breakthrough, identifying C4A and ApoB as promising biomarkers [82]. This development marks a new era in the diagnostic approach to this complex mental disorder [83], offering insights into underlying pathophysiological mechanisms and facilitating early detection and targeted therapeutic strategies.

The β-casomorphin-7 aptamer plays a crucial role in detecting β-casomorphin, representing a significant advancement in diagnostic approaches for autism spectrum disorder (ASD) [84]. This biomarker-based technique offers a more objective and potentially earlier identification of ASD, surpassing traditional behavioral assessments and enabling timely, personalized interventions.

The therapeutic use of the VEGF aptamer in spinal cord injuries demonstrates a novel approach to enhancing recovery [85]. By promoting angiogenesis, this treatment addresses a critical aspect of healing spinal cord injuries, potentially accelerating tissue regeneration and improving functional outcomes in a condition historically challenging to treat.

The RB006 aptamer's specific binding to Factor IXa in ischemic stroke management provides a precise method for regulating clot formation [86], reducing the risk of systemic bleeding—a significant concern with broad anticoagulants. This targeted intervention may improve stroke treatment outcomes by directly addressing vascular occlusion.

The P2X7 aptamer's targeting of the P2X7 receptor in chronic pain represents a novel approach to pain management [87]. Modulating this receptor crucial in pain signaling offers a promising avenue for significantly reducing pain perception, particularly beneficial in conditions with complex pain management challenges and limited treatment options.

Aptamers also play a crucial role in neurodegenerative disorders like Alzheimer's and Parkinson's diseases. The beta-amyloid aptamer in Alzheimer's disease specifically targets and inhibits the aggregation of beta-amyloid plaques [88], potentially slowing disease progression. In Parkinson's disease, the alpha-synuclein aptamer shows promise by inhibiting alpha-synuclein aggregation [89], a departure from current symptomatic treatments toward disease-modifying approaches.

Versatile Applications of Aptamers Across Therapeutics, Diagnostics, and Environmental Monitoring

Aptamers are highly versatile and promising tools with a wide range of applications in fields such as therapeutics, diagnostics, environmental monitoring, and biosensing. Aptamers, exemplified by Pegaptanib, have significantly transformed the therapeutic approach to age-related macular degeneration by precisely targeting pathologic proteins [100, 101]. They offer high specificity and sensitivity in diagnostic tests for conditions such as cancer or infectious diseases [102].

Table 4 This table offers a brief overview of several disorders, the relevant aptamers used for their potential treatment or biomarker identification, their mechanisms of action, and the associated scientific references. Each row specifies an aptamer's role in modulating or detecting a particular disorder, as supported by the referenced studies.

Disorder	Aptamer	Mechanism of Action	References
Schizophrenia	**X-Aptamer**	**Identifies C4A and ApoB in Blood as Potential Markers**	**[82]**
Autism Spectrum Disorder	β-casomorphin-7 (BCM-7) Aptamer	detection of the β-casomorphin (BCM-7) as a promising biomarker of autism disorder	**[84]**
Spinal Cord Injury	VEGF Aptamer	Binds to Vascular Endothelial Growth Factor (VEGF), promoting angiogenesis and recovery	**[85]**
Ischemic Stroke	RB006 Aptamer	Binds to Factor IXa, reducing clot formation	**[86]**
Chronic Pain	P2X7 Aptamer	Binds to P2X7 receptor, reducing pain signaling	**[87]**
Prion Diseases	PrP Aptamer	Binds to prion protein, reducing its pathological conformation	**[90]**
Multiple Sclerosis	MBP-1 Aptamer	Binds to myelin basic protein, modulating immune response	**[91]**
Huntington's Disease	guanine-rich aptamers	showing high efficacy in modulating the functions of the mutated protein	**[92]**
Diabetic Neuropathy	NfL Aptamer	Binds to Neurofilament Light, a biomarker for nerve damage, enabling monitoring and potential therapeutic interventions	**[93]**
Fragile X Syndrome	FMRP Aptamer	Binds to Fragile X Mental Retardation Protein, potential therapeutic applications for genetic disorders	[94]
Myasthenia Gravis	Acetylcholine Receptor Aptamer	Binds to Acetylcholine Receptor, potentially reducing autoantibody-mediated damage	[95]
Dementia	BACE1 Aptamer	Inhibits BACE1 enzyme, reducing amyloid-beta production	**[96]**
Alzheimer's Disease	beta-amyloid Aptamer	Binds to beta-amyloid, inhibiting its aggregation	**[88]**
Parkinson's Disease	alpha-synuclein Aptamer	Binds to alpha-synuclein, inhibiting its aggregation	**[89]**
Amyotrophic Lateral Sclerosis (ALS)	SOD1 Aptamer	Binds to superoxide dismutase 1 (SOD1), reducing its aggregation	**[97]**
Stroke/Ischemia	NMDA receptor Aptamer	Binds to NMDA receptors, reducing glutamate-induced neurotoxicity	**[98]**
Tourette Syndrome	Dopamine D2 Receptor Aptamer	Binds to Dopamine D2 Receptor, potential for use in adjusting dopaminergic signaling	[99]

Abbreviations: C4A: Complement Component 4A, Apo: Apolipoprotein, PrP: Prion Protein, MBP: Myelin Basic Protein, NfL: Neurofilament Light, FMRP: Fragile X Mental Retardation Protein, BACE: Beta-Site APP Cleaving Enzyme, SOD: Superoxide Dismutase, NMDA: N-Methyl-D-Aspartate.

In environmental protection, aptamers play a significant role by cost-effectively detecting contaminants like mercury, ensuring food safety through the identification of foodborne pathogens [103]. Furthermore, they serve as carriers for transporting therapeutic agents to specific cells, thereby reducing overall toxicity in cancer therapy [104].

Limitations and Delivery Methods of Aptamers in Therapeutic Applications

Aptamers, despite being recognized for their specificity and potential in targeted therapies, encounter several fundamental limitations that hinder their clinical applicability. One major consideration is the stability of these entities in the biological environment [105]. Aptamers, especially those based on RNA, are susceptible to degradation by nucleases in the body's fluids [106], leading to a notable reduction in their therapeutic effectiveness. To address this problem, significant efforts have been focused on chemical modifications, including the integration of unnatural nucleotide analogs, which can provide resistance against nucleases [107]. Nevertheless, these modifications may unintentionally impact the aptamer's binding affinity or specificity, as well as increase production costs, which could hinder the feasibility of large-scale manufacturing and widespread clinical implementation. The specificity of aptamers poses challenges despite being a defining characteristic. In the complex and diverse environment of the human body, it is important to consider the potential for aptamers to bind to molecules that are not their intended targets or to similar regions on different cells. The lack of specificity in binding raises concerns regarding potential off-target effects [108], which may result in unfavorable outcomes. It is crucial to carefully optimize the structure of the aptamer and conduct thorough in vivo testing in order to minimize these risks. Aptamers are commonly considered to have lower immunogenicity compared to protein-based therapeutics with respect to their immunogenic properties [109]. Nonetheless, the immune response may still occur to some extent, particularly when the administration is repeated or prolonged. Minimizing immunogenic responses is crucial in the development of aptamers, especially for long-term therapeutic use. In the field of delivery methods, various innovative strategies have been developed to improve the effectiveness of aptamer therapeutics. Nanoparticle-based delivery systems have become increasingly important due to their ability to protect aptamers from enzymatic degradation [110] and facilitate targeted and controlled release. Nanoparticles can be customized to target specific cell types or tissues, thereby improving the therapeutic effectiveness of aptamers. Conjugation techniques involve attaching aptamers to different carriers such as lipids, polymers, or other biological molecules [111]. This strategy aims to enhance the bioavailability and prolong the half-life of aptamers. Conjugates can enhance tissue penetration, particularly in dense or inaccessible regions, and assist in targeting specific cellular sites for aptamer delivery. Local administration of aptamers, particularly in diseases such as cancer, provides a means to enhance therapeutic efficacy at the specific target site while minimizing systemic exposure [112]. This approach is particularly applicable to solid tumors, as localized treatment can greatly enhance effectiveness. Exosome-based systems offer a promising approach for delivering aptamers [113, 114], representing a new and innovative method in the field of delivery methods. Exosomes, being natural carriers, can encapsulate aptamers, thereby protecting them from degradation and facilitating precise delivery to particular cell types. Hydrogel-based delivery systems are gaining attention in regenerative medicine due to their capacity for localized and sustained release of aptamers [115].

CONCLUSION

Aptamers, single-stranded nucleic acids, show immense promise in diagnosing and treating a variety of diseases, including oncology, respiratory conditions like asthma and chronic obstructive pulmonary disease (COPD), and neurological disorders. These compounds

demonstrate high specificity and affinity towards diverse targets, enabling precise interventions with reduced toxicity. Aptamers hold potential for early cancer detection and immunotherapy. In respiratory diseases, targeting specific cytokines offers therapeutic advantages over conventional treatments.

Preliminary research suggests aptamers may also influence neurological disorders by modulating gut-brain communication. However, challenges remain in ensuring their stability, kinetics in living organisms, and cost-effectiveness. Further research is essential to optimize these parameters and validate the long-term effectiveness and safety of aptamers. Despite these challenges, aptamers possess significant versatility and potential to profoundly impact healthcare, warranting continued investigation.

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التطورات والعقبات المرتبطة بالاستراتيجيات العلاجية والتشخيصية المعتمدة على الأبتاميرات عبر مجالات طبية متنوعة: دراسة شاملة ومعمقة

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ملخص

تُعتبر الأبتاميرات، وهي جزيئات من الحمض النووي الريبوزي المنقوص الأكسجين أو الحمض النووي الريبوزي، أدوات مهمة تكتسب شهرة متزايدة في مجالات التشخيص والعلاج في تخصصات طبية متنوعة مثل علم الأورام، الأمراض الرئوية، والاضطرابات العصبية. تقدم هذه الدراسة تقييمًا شاملًا للتطورات الأخيرة والتحديات التي تُواجهها مجالات تطبيقات الأبتاميرات. لقد أظهرت الأبتاميرات فعالية في الكشف المبكر عن السرطان وفي التوصيل الهدفي للعقاقير، مُقللةً من التأثيرات الجانبية. وتُبدي الأبتاميرات إمكانات كبيرة في إدارة الربو ومرض الانسداد الرئوي المزمن، من خلال استهداف السيتوكينات وتنظيم الاستجابة الالتهابية. تُقدم وسائل جديدة لإدارة الاضطرابات العصبية بفضل تأثيرها على علاقة الأمعاء والدماغ. رغم هذه الفوائد، لا تزال هناك تحديات مُستمرة تتعلق بالاستقرار الجزيئي وآليات التوصيل والجدوى الاقتصادية. تسلط هذه الدراسة الضوء على الجوانب المُتعددة لتطبيقات الأبتاميرات في التشخيص والعلاج، وتقدم نظرة شاملة للبحوث المستقبلية المُحتملة.

الكلمات الدالة: العلاج بالآبتامير؛ آبتاميرات التشخيص؛ تعديل المناعة؛ علاقة الدماغ–الأمعاء؛ مؤشرات خاصة بالأورام.

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تاريخ استلام البحث 2023/10/21 وتاريخ قبوله للنشر 2024/1/30.

Evaluating the Validity and Reliability of Questionnaires Measuring Knowledge, Attitudes, and Practices Towards Antibiotic Resistance Among Youths: A Systematic Review Protocol

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ABSTRACT

This protocol for a systematic review describes the methodology for assessing the validity and reliability of questionnaires used in studies on antibiotic resistance among youths. It also seeks to evaluate the methodological quality of these studies in terms of their ability to influence youths' knowledge, attitudes, and practices (KAP) regarding antibiotic resistance, as measured by the identified questionnaires. The review will include an exhaustive literature search spanning 2013 to 2023 using key databases and grey literature sources. Data from eligible studies will be extracted regarding sample characteristics, methodological quality, and questionnaire validity and reliability metrics. The participants will be categorized into secondary school, high school, and undergraduate students. The review thoroughly evaluates the instruments' psychometric features, including face validity, internal consistency, test-retest reliability, construct validity, and hypothesis testing. Moreover, the protocol thoroughly examines the methodology and approach employed in the encompassed research, specifically emphasizing the educational setting and its impact on the efficacy of interventions to combat antibiotic resistance. The classification of individuals based on their educational stages enables a comprehensive evaluation of the effectiveness of KAP questionnaires in various educational contexts. A meta-analysis will be performed to quantify the cumulative effects of studies. The systematic review is anticipated to provide valuable insights into the validity and reliability of questionnaires used in antibiotic resistance studies focusing on youths. By evaluating the methodological quality of these studies, this review intends to contribute to the development of standardized measurement instruments and to enhance our understanding of how interventions impact youths' KAP related to antibiotic resistance.

Keywords: Questionnaires, Knowledge, Attitudes, Practices, Antibiotic Resistance, Youths, Systematic Review, Protocol
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1. INTRODUCTION:

Antimicrobial Resistance (AMR) refers to the development of bacterial resistance to antibiotics, which were once efficacious in treating infections. This can occur naturally or because of overuse or misuse of antibiotics [1]. Antibiotic resistance is a significant public health issue that affects people across all age cohorts, including youths and adolescents. Prolonged illness, heightened mortality rates, and escalated healthcare expenditures ensue, resulting in extended hospitalizations for afflicted individuals [2][3].

Improper management of antibiotic drugs, such as prematurely discontinuing the treatment and utilizing remaining doses [4], among the younger generation is a significant concern [5]. The utilization of antibiotic therapies is disproportionately higher among younger

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patients compared to middle-aged populations [6]. The level of understanding among adolescents and young adults aged 14 to 24 regarding medical conditions effectively treatable with antibiotics is relatively lower compared to other age cohorts [7].

According to a comprehensive survey conducted in Europe, individuals between the ages of 15 and 24 exhibit the greatest rates of antibiotic usage. Moreover, compared to other demographic cohorts, this age group is more likely to employ antibiotics to treat upper respiratory tract infections (URTIs) [8]. Moreover, a study carried out in the United Kingdom found that persons in the younger age group (15-34 years) have worse levels of comprehension of AMR and the proper handling of self-limiting infections compared to older age groups [9]. Conversely, a cross-sectional study conducted in Palestine revealed a significant correlation between greater education, younger age, gender, occupation, high monthly income, and strong knowledge with a positive attitude toward antibiotic use [10].

This systematic review will evaluate the accuracy and consistency of questionnaires used to measure knowledge, attitudes, and behaviors (KAP) about antibiotic resistance among young people, specifically focusing on educational levels such as high school, secondary school, and undergraduate students. Previously, a comprehensive systematic review focused on the public without providing a detailed analysis based on their educational levels. In contrast, the current review takes a more specific perspective by focusing on the youth population and classifying them based on their educational status, including high school, secondary school, and undergraduate students [11]. The study largely concentrated on the methodology and tactics utilized in existing population-based surveys about antibiotic knowledge and awareness of antimicrobial resistance (AMR). While the referred research did not specify a quantitative data synthesis method, this systematic review presents a meta-analysis strategy to synthesize data and construct visual representations using forest plots. In addition, the present research employs the Cochrane Risk for Bias instrument to assess the possible presence of prejudice in the included studies. It is worth noting that the prior systematic review did not mention the utilization of this technique for assessing the risk of bias [11]. The differentiations underscore the unique focus, goals, and methodologies of systematic reviews, which hold the potential to significantly enhance comprehension of antibiotic resistance among youths in diverse settings.

According to the World Health Organization, youths between the ages of 15 and 24 undergo fast physical, cognitive, and emotional development [12]. This period is critical for their overall well-being but is also associated with high death rates, illness, and injury [13]. In his book on adolescence at the start of the twentieth century, Stanley Hall provided an informal definition of adolescence as the developmental phase that spans from 14 to 24 years [14]. The adolescent population is a significant demographic group in a nation and requires immediate attention. As current and future leaders, adolescents drive economic, social, and cultural progress [15]. According to a study conducted in England, adolescents have limited comprehension of antibiotics and the implications associated with antimicrobial resistance (AMR) [8]. The study additionally indicated that adolescents lacked interest in antibiotics, resulting in limited discourse among peers and educators. This lack of participation ultimately contributed to the comparatively low level of understanding regarding antibiotic resistance. Interventions promoting expert-driven behavioral change, effective communication, education, and training that enhance awareness and comprehension of rational antimicrobial use are strongly recommended. Additionally, restrictions on the distribution of antibiotics should be imposed by law [16].

The term "youth" gained popularity around the time of the United Nations' inaugural International Youth Year in 1985, coinciding with adolescence. The understanding of

youth generally encompasses those aged between 15 and 24 years. However, as articulated during the related international congress, the Barcelona Statement conceptualized youth as a social construct devoid of specific age parameters [17]. Most definitions of the more recently introduced terms young adulthood and emerging adulthood fall between 18 and 26 [18][19][20].

Antibiotic resistance interventions often employ questionnaires to measure their effectiveness, particularly in changing KAP among youths. However, the validity and reliability of these questionnaires have not been comprehensively assessed. This gap in the literature makes it challenging to compare the effectiveness of different interventions and to develop standardized measurement tools for future studies. Despite the crucial significance of this matter, there is a lack of complete assessment of the soundness and reliability of surveys used for gauging KAP of youths about antibiotic resistance. Questionnaires are frequently utilized in interventions that target modifying KAP within this population. The lack of a comprehensive assessment poses challenges in assessing the efficacy of various interventions, hence impeding the establishment of standardized measurement instruments for future research endeavors.

Given this context, the research question for this systematic review is as follows: "What are the validity and reliability of questionnaires used in studies evaluating youths' KAP regarding antibiotic resistance, and how effective are these studies at altering youths' KAP as measured by these questionnaires?"

The lack of standardized measurement tools in antibiotic resistance interventions hampers the ability to compare effectiveness and develop evidence-based strategies. This systematic review seeks to address this deficiency by identifying valid and trustworthy surveys, guiding the design of future interventions, and contributing to antibiotic stewardship among youths. Understanding the validity and reliability of these surveys is critical for creating successful antibiotic resistance education campaigns for teenagers. A systematic review is required to collect and assess existing literature, fill the current gap, and facilitate future research and intervention initiatives.

2. THE REVIEW:

2.1. Aims:

This study aims to i) conduct a comprehensive analysis to identify and assess the validity and reliability of questionnaires employed in research studies that assess youths' KAP about antibiotic resistance, and ii) evaluate the methodological quality of the studies that used these questionnaires and the effectiveness of these studies in changing youths' KAP about antibiotic resistance, as measured by the identified questionnaires. The scope of this investigation will encompass scholarly publications released within the timeframe spanning from 2013 to 2023.

2.2. Methodology:

2.2.1. Design:

A systematic review that aims to evaluate the validity, reliability, and methodological quality of research utilizing questionnaires.

2.2.2. Search Strategy:

To ensure a thorough and targeted exploration of the existing literature, a systematic search will be conducted across four electronic databases: PubMed, CINAHL, Scopus, and Google Scholar. These databases have been chosen based on their pertinence to research in medicine and healthcare. The search scope will be restricted to publications published between January 2013 and December 2023 to encompass the most up-to-date and pertinent research outcomes.

The selection of search terms has been carefully chosen to correspond with the study inquiry. Boolean operators will be employed to enhance the precision and specificity of the search. The study will employ the following sets of search terms: (“Knowledge” OR “Attitudes” OR

"Practices" OR "Behaviors" OR "Awareness") AND ("Adolescents" OR "Teens" OR "Youth" OR "Teenagers") AND ("Questionnaires" OR "Surveys") AND ("Antibiotic Resistance" OR "Drug Resistance" OR "AMR" OR "Antimicrobial Resistance") NOT ("Antivirals" OR "Antifungals" OR "Animal Studies" OR "topical antimicrobials") AND ("Validity" OR "Reliability" OR "Measurement" OR "Assessment Tools").

A set of specific restrictions will be implemented to achieve a more refined selection of search results. Firstly, only articles that have been published in the English language will be considered for inclusion. Studies involving antiviral, antifungal, animal experimentation, or topical antimicrobial agents will be omitted. This detailed search strategy aims to achieve a high level of specificity, guaranteeing that only the most relevant studies are incorporated into the review.

2.2.3. Study Selection:

Inclusion Criteria

- Original research articles.
- Studies assessing KAP about antibiotic resistance.
- Involved youths between 14 – 24 years old.
- Manually administered or interviewer-administered questionnaire studies.
- Published between 2013 – 2023.

Exclusion Criteria

- Studies not published in English.
- Studies focus on topical antimicrobials.
- Investigations that employ data collection methods other than self-administered or interviewer-administered questionnaires.
- Research articles that fail to provide empirical data or methodological details concerning the utilized questionnaire's validity and/or reliability.
- Narrative reviews or editorial papers as they do not contain a methods section.

2.2.4. Data Extraction

Data from each eligible study will be extracted systematically using a standardized form. The full citation of the paper, including author(s) and year of publication, as well as the name of the questionnaire or instrument employed, will be extracted. The location of the study will also be mentioned. The study design will be recorded, indicating whether the study is a cross-sectional, cohort, or randomized controlled trial. Information on the sample size and response rate will be provided if available. The characteristics of participants, such as their age range and gender distribution, will be discussed. Specific variables or areas the questionnaire aims to measure, such as antibiotic resistance KAP, will be identified. The data collection method, whether self-administered or conducted by an interviewer, will be indicated. Outcomes from the study will be extracted, including any metrics or scales used for validity and reliability, such as Cronbach's alpha or test-retest reliability. The following tools—construct validity, internal consistency, reliability, face validity, and hypothesis testing—will be evaluated for validity and reliability.

Quality Assessment

To evaluate the efficacy of the instruments utilized in the research under consideration, we will adhere to the guidelines and templates provided by COSMIN (Consensus-based Standards for selecting health Measurement Instruments), as outlined by Mokkink (2018). The methodology is well acknowledged for its strong capacity to assess the psychometric characteristics of outcome measures. Instruments will be assessed within three primary categories: reliability, validity, and responsiveness.

The reliability domain encompasses an assessment of measurement inaccuracy, internal consistency, and reliability. Internal consistency pertains to the degree to which elements within a measurement instrument are interconnected according to the one-dimensionality

assumption [21]. Reliability can be understood as the measurement of consistency or stability over time. One commonly used method to assess reliability is test-retest reliability, which consists of conducting two separate measurements of the same construct and analyzing the degree of concordance between the results. This can be quantified using statistical techniques such as the intraclass correlation coefficient (ICC) [21].

Content validity (including face validity), structural validity, hypothesis testing for construct validity, cross-cultural validity, and criterion validity are all components of the validity assessment's domain [18]. Structural validity refers to the extent to which scores accurately represent the fundamental structure of the construct being evaluated [21].

The review process will be structured into three tables to facilitate a systematic study. In the first table, the validity and dependability of the questionnaires utilized in the studies will be assessed, evaluating each questionnaire's psychometric performance. This table will show how well the questionnaires measure youths' antibiotic resistance KAP.

The second table will summarize the studies' methodology, sample characteristics, education levels (secondary school, high school, and undergraduate students), and critical conclusions. This table will compare the studies' methodologies, highlighting their strengths and weaknesses, and help explain study diversity. The education level is essential since it considerably impacts youths' understanding of antibiotic resistance. Data will be analyzed individually for secondary school, high school, and undergraduate students by categorizing the studies based on participants' educational backgrounds. This classification helps to measure the efficacy of interventions at various educational stages.

The third table will cover the themes and questions regarding KAP. The purpose of this table is to offer a comprehensive analysis of the inquiries and topics about KAP surrounding antibiotic resistance. By categorizing issues and themes, a more comprehensive understanding of the principal areas of emphasis within the encompassed subjects can be attained. Additionally, this table will provide information regarding the nature of the question, such as whether it is a multiple-choice or a Likert scale question, as well as its correspondence with the KAP components. Furthermore, we will consider the participants' educational levels to determine whether the questions and subjects differ depending on their educational backgrounds.

Practices incorporating educational attainment levels within these tables will augment the comprehensiveness of our analysis, enabling us to derive more significant insights into the influence of educational stages on the knowledge, attitudes, and practices of adolescents concerning antibiotic resistance. This technique aligns with our objective to offer significant insights into the validity and reliability of surveys across diverse educational environments.

2.2.5. Data Synthesis:

The primary approach for data synthesis in this systematic review will involve the utilization of meta-analysis. This statistical technique will be employed to combine the results from the included research, and a forest plot will be used to summarize the collective effects visually and quantitatively. The quantitative approach utilized in this research will begin with calculating effect sizes, which may include odds ratios for categorical data or mean differences for continuous data. Additionally, matching confidence intervals will be determined for each study. The heterogeneity among research will be evaluated by applying statistical tests, such as Cochran's Q and the I^2 statistic, to measure the extent of variability observed in the results of different studies.

A forest plot will be utilized to present the meta-analysis results comprehensively. The effect sizes and confidence intervals for each study incorporated in the analysis will be presented in this plot, facilitating a

comprehensive scrutiny of the findings. Additionally, the forest plot will summarize the overall effect, offering a concise representation of the collective findings. When encountering significant heterogeneity, researchers will perform subgroup analysis and sensitivity studies to investigate potential factors contributing to the observed variability. In addition, assessing publication bias will involve the utilization of methodologies such as funnel plots and statistical tests.

A forest plot will be a highly effective visual instrument for communicating the findings, providing valuable information regarding the overall effect's direction and magnitude and the observed variability among the various studies. This methodology will substantially contribute to compiling evidence about antibiotic resistance among youths, ensuring the findings are clearly presented and can be effectively interpreted.

2.2.6. Risk for Bias:

The Cochrane Risk of Bias instrument will be applied to assess the potential for bias in the included studies. As moderator factors, additional potential sources of bias, including funding and publication status, will also be investigated.

2.2.7. Ethical Considerations

This review does not entail direct interactions with human or animal subjects. As such, no special ethical approvals or informed consent are required. The review focuses exclusively on the analysis of previously published studies and the synthesis of available data. Given the nature of this research, which involves the examination of existing research outputs, no special ethical considerations or permissions are required.

3. DISCUSSION AND CONCLUSIONS

This protocol for a systematic review outlines the methods for analyzing the validity and reliability of questionnaires used in research on antibiotic resistance among adolescents. The methodological quality of these studies in terms of their capacity to reflect the KAP of young people in relation to antibiotic resistance will be highlighted. Information regarding the quality of the methodologies employed, the characteristics of the samples, and the questionnaires' validity and reliability metrics will be obtained from studies appropriate for the present analysis.

The present analysis will encompass an assessment of the construct validity, face validity, internal consistency, test-retest reliability, and overall reliability of the instruments. A meta-analysis will quantify the combined impact of all the research. This review proposes to contribute to the development of standardized assessment tools and to expand our understanding of how interventions affect young people's knowledge, attitudes, and behaviors (KAP) linked to antibiotic resistance by assessing the methodological quality of the research.

This protocol is expected to have some inherent limitations. Systematic reviews and meta-analyses are constrained in their ability to calculate effect sizes by the number of available studies on the topic, the level of detail provided in the study reports regarding interventions, and the reported data types. All eligible studies will be incorporated, and the authors of primary studies that fail to provide adequate data for effect size calculation will be contacted.

Conflict of interest statement

The authors assert that they have no conflicting interests.

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تقييم صحة وموثوقية استبيانات قياس المعرفة والمواقف والممارسات تجاه مقاومة المضادات الحيوية بين الشباب: بروتوكول مراجعة منهجية

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ملخص

يصف هذا البروتوكول للمراجعة المنهجية منهجية تقييم صحة وموثوقية الاستبيانات المستخدمة في الدراسات حول مقاومة المضادات الحيوية بين الشباب. ويسعى أيضًا إلى تقييم الجودة المنهجية لهذه الدراسات من حيث قدرتها على التأثير على معارف الشباب ومواقفهم وممارساتهم (KAP) فيما يتعلق بمقاومة المضادات الحيوية، كما تم قياسها من خلال الاستبيانات المحددة. باستخدام قواعد البيانات الرئيسية ومصادر الأدبيات الرمادية، ستتضمن المراجعة بحثًا شاملاً في الأدبيات يمتد من عام 2013 إلى عام 2023. وسيتم استخراج البيانات من الدراسات المؤهلة فيما يتعلق بخصائص العينة، والجودة المنهجية، ومقاييس صلاحية الاستبيان وموثوقيته. سيتم تصنيف المشاركين إلى طلاب المدارس الثانوية والمدارس الثانوية والطلاب الجامعيين. تستلزم المراجعة إجراء تقييم شامل للميزات السيكو مترية للأدوات، بما في ذلك تقييم صلاحية الوجه، والاتساق الداخلي، وموثوقية الاختبار وإعادة الاختبار، وصلاحية البناء، واختبار الفرضيات. وعلاوة على ذلك، فإن البروتوكول يدرس بدقة المنهجية والنهج المستخدم في البحث الشامل، مع التركيز على وجه التحديد على البيئة التعليمية وتأثيرها على فعالية التدخلات لمكافحة مقاومة المضادات الحيوية. يتيح تصنيف الأفراد بناءً على مراحلهم التعليمية إجراء تقييم شامل لفعالية استبيانات KAP في العديد من السياقات التعليمية. لتحديد الآثار التراكمية للدراسات، سيتم إجراء التحليل التلوي. ومن المتوقع أن توفر المراجعة المنهجية رؤى قيمة حول صحة وموثوقية الاستبيانات المستخدمة في دراسات مقاومة المضادات الحيوية التي تركز على الشباب. ومن خلال تقييم الجودة المنهجية لهذه الدراسات، تهدف هذه المراجعة إلى المساهمة في تطوير أدوات قياس موحدة وتعزيز فهمنا لكيفية تأثير التدخلات على المعرفة والأفكار والممارسات لدى الشباب فيما يتعلق بمقاومة المضادات الحيوية.

الكلمات الدالة: مقاومة المضادات الحيوية، الاستبيانات، الشباب، المعرفة، المواقف، الممارسات، مراجعة منهجية، بروتوكول.

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Association between ABO Blood Group System and the Severity of COVID-19 in the West Bank: A Case-control Study

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ABSTRACT

Introduction: Several studies have examined risk factors for COVID-19, and there have been conflicting results regarding whether blood type influences the risk of COVID-19. Therefore, this study aimed to determine the association between the ABO blood group system and the severity of COVID-19 in the West Bank of Palestine.

Methods: A case-control study design was used, consisting of 169 cases and 169 controls who had undergone COVID-19 PCR testing in March 2021 in the West Bank, stratified according to their test results. A self-administered questionnaire in Arabic was used, which included the following parts: socio-demographic data, smoking status, seasonal flu, medical history of COVID-19, and the experience of COVID-19 cases. Both descriptive and analytical analyses were carried out. Binary logistic regression (Enter model) was used in the multivariate analysis to demonstrate the odds ratio.

Results: The study included 338 participants (169 cases and 169 controls) with a 95% response rate. Among the participants, 46.4% lived in the northern region (Nablus, Jenin, Qalqilya, Salfit, Tubas, Tulkarm). Blood group B was found to be more protective against COVID-19 than blood group A (AOR=0.40, CI=0.223-0.718). Blood group AB was also more protective against COVID-19 than blood group A ($P < 0.05$, AOR=0.316, CI=0.143-0.698). There was no statistically significant difference between blood group A and blood group O concerning the severity of symptoms or the composite outcomes of COVID-19.

Conclusion: There is an association between the ABO blood group and the risk of COVID-19. Nonetheless, the precise mechanism and severity of the effect vary, necessitating further investigation through larger and more intensively controlled studies.

Keywords: Palestine, Covid-19, blood type, severity of symptoms.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a pandemic respiratory infection caused by a novel coronavirus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [1]. On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a pandemic. This extremely infectious virus can be asymptomatic or present with mild to severe symptoms, and it may be life-threatening to high-risk individuals such as the elderly, patients with immunosuppression, and those with underlying medical conditions such as cardiovascular disease and cancer [2]. The virus is transmitted from person to person among both symptomatic and asymptomatic individuals [3].

Researchers have explored the mechanisms of viral entry into human hosts via endocytosis, histo-blood group antigen, cell surface receptors, and acetylcholine receptors in respiratory diseases. There is documented evidence of auto-antigenicity and hematological problems, such as the agglutination of red

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blood cells by the serum from infected individuals [4]. In 1901, Karl Landsteiner discovered the ABO blood group system, which encouraged researchers to investigate the connection between the ABO blood group system and various diseases. It has been suggested that many bacterial and viral infections, such as Helicobacter pylori, norovirus, hepatitis B virus, and severe acute respiratory syndrome coronavirus (SARS-CoV), are associated with the ABO blood group system [5].

Moreover, understanding the link between illnesses that have resulted in pandemics and blood types might be a helpful risk factor for predicting outcomes and developing effective strategies to combat disease transmission in relation to blood group distributions [6]. There are significant uncertainties regarding blood type and MERS infection during the severe acute respiratory syndrome coronavirus (SARS-CoV) episode that began in late 2002, along with risk factors that may exacerbate disease severity and progression [7]. This has often led to increased symptom severity and raised more questions than answers regarding susceptibility to COVID-19, which has recently been linked to ABO blood groups in patients [7].

This study aimed to assess the association between blood group and the severity of COVID-19 in Palestine.

METHODOLOGY

Sampling method

The study was conducted across all governorates in the West Bank that had tested for COVID-19 from March 1, 2021, to March 31, 2021 (Bethlehem, Hebron, Jericho, Ramallah, Nablus, Jenin, Qalqilya, Salfit, Tubas, Tulkarm, and Jerusalem) using a case-control study design and stratified systematic sampling method. There is an electronic Excel sheet program called the District Health Information System for COVID-19 (DHIS), which is used by all health directorates to enter the contact information of patients conducting PCR tests along with the test results.

First, the researcher obtained the electronic Excel sheet file for all governorates of the West Bank from the Palestinian Ministry of Health. Then, the data were stratified according to their COVID-19 PCR results into two groups: the first stratum was positive, and the second stratum was negative. After that, the researcher systematically selected the patients from each stratum using a kth interval by dividing the entire population size by the desired sample size [8].

The following operational definitions were enrolled in the study:

The Risk: The susceptibility to have positive result of COVID-19 PCR.

The severity of symptoms: They are categorized into: (Mild, Moderate, Severe, and Critical illness) which defined according to WHO as the following:

Mild symptoms: These include low-grade fever (not more than 100 degrees Fahrenheit), cough, sore throat, malaise, headache, muscle pain, congestion or runny nose, loss of taste or smell, and diarrhea without shortness of breath (9).

Moderate symptoms: A patient might start to show evidence of lower respiratory disease, fever of about 101-102 degrees Fahrenheit, and chills with repeated shaking, but they still have good oxygenation on room air in addition to any of the mild symptoms [9].

Severe symptoms: These include shortness of breath (generally breathing quickly over 30 times a minute), chest discomfort, confusion/unresponsiveness and oxygenation level is less than 94%, and which generally means that the patient needs supplemental oxygen [9].

Critical illness: These include the signs of respiratory failure and the need for a breathing machine and patients may experience other organ dysfunction and shock which require to intensive care unit (9).

COVID-19 outcomes: They are categorized into: (Require mechanical ventilation, Admission to intensive care unit, Complications post COVID-19).

Inclusion Criteria

Cases were defined by the final nasal or pharyngeal swab is a positive result, only alive patients were selected, have contact information at the Ministry of Health register file as the phone number, know their ABO Blood group and have signs and symptoms.

The controls who have tested negative result for the COVID-19 PCR from the health electronic data from 1/3/2021 until 31/3/2021. As it should be the final nasal or pharyngeal swab is a negative result, only alive patients were selected, have contact information at the Ministry of Health register file as the phone number, know their ABO Blood group and not infected with COVID-19 previously.

Exclusion Criteria

The selected controls were excluded if they had a positive result before the last one, refused to participate in the study, or did not know their ABO blood group. The selected cases were excluded if they were asymptomatic, had died, were in critical condition and unable to answer the questionnaire, did not know their ABO blood group, or refused to participate in the study. In our study, 9 cases and 9 controls were excluded because 13 subjects did not know their blood group and 5 refused to participate.

Sample size determination

The sample size for the case-control study was determined with 95% confidence. The association between the ABO blood group and COVID-19 has 80% power and an estimated odds ratio of 2.0, with a case-control ratio of 1:1. A hypothetical assumption of risk (20%) was applied to the control group, as stated in the literature for a similar study conducted in Saudi Arabia. The distribution of blood groups between Saudi Arabia and Palestine is similar, with the most prevalent blood groups being A and O [10]. This generated a total sample size of 372 for the study. 34 pairs of cases and controls were excluded: 16 for the piloting study and 18 because they did not meet the selection criteria, resulting in a total sample size of 338.

Data Collection Procedure

Data collection took place between March 25, 2021, and April 25, 2021. The health electronic files available for those with a positive or negative PCR test were used, and participants were contacted via phone for about ten minutes. Those who were unable to answer the questions by phone were interviewed in their hospitals or COVID-19 centers if possible.

Study Instrument

A questionnaire in Arabic was modified and developed based on the study's aim and objectives and its conceptual framework. The smoking status section was adopted from the WHO Stepwise questionnaire with minor modifications to align with the study factors. It consisted of the following parts: socio-demographic data, smoking status, seasonal flu, medical history of PCR COVID-19, and the experience of COVID-19 cases. The questionnaire took approximately 10 minutes to complete.

Pilot study of the questionnaire

The questionnaire was validated and piloted after its development. The pilot study involved 16 COVID-19 patients and was validated by 8 experts in epidemiology, scientific research, and public health. The reliability for different sections ranged between 72.0 and 92.0.

Data Analysis

Both analytical and descriptive analyses were performed. Descriptive statistics were used to display frequencies and percentages for categorical variables, as well as means and standard deviations for continuous variables. The Chi-square test and multivariate analysis utilized binary logistic regression (Enter Model). Our primary measure of association was the Adjusted Odds Ratios (AORs) with 95% confidence intervals (95% CI). P-values less than 0.05 were considered significant.

Ethical consideration

Ethical approval was obtained from the Al Quds University Ethical Research Committee (REC). Additional ethical approval and consent to conduct the study were obtained from the Palestinian Ministry of Health. The consent form was signed by the participants.

RESULTS

Our study comprised 338 participants (169 cases and 169 controls) with a 95% response rate. Most of our participants (46.4%) were living in the northern region (Nablus, Jenin, Qalqilya, Salfit, Tubas, Tulkarm), and 60.7% were married. The mean age was 38 years (the youngest was 14 years old, and the oldest was 84 years).

Most participants' blood group was "O" (37.9%), followed by "A" (36.7%); 13.6% had blood group "B," and 11.8% had blood group "AB." Regarding the rhesus factor (RH), the majority of participants were RH positive (80.2%), while 19.8% were RH negative. Univariate analysis revealed no statistically significant difference between the cases and controls according to sociodemographic characteristics except for the blood group (P.value <0.05), as shown in Table 1.

ABO Blood group and the severity of COVID-19

The study revealed no significant difference between the ABO blood group and the severity of symptoms or the duration of symptoms. Additionally, the results showed no association between the ABO blood group and the outcome of COVID-19 (P > 0.05), as shown in Table 2.

Multivariate analysis

Multivariate analysis showed significant differences between blood group "A" and other blood groups. Based on AOR and CI, the results showed that blood group "B" is more protective against COVID-19 (AOR= 0.40, CI= 0.223-0.27). There was also a significant difference between blood groups "A" and "AB," with "AB" being more protective against COVID-19 by 31.6% (P <0.05, AOR= 0.316, CI= 0.143-0.698).

Table 1: Univariate analysis for cases and controls according to their characteristics

Variables		Cases (N%)	Controls (N%)	P value
Sex	Male	86 (50.9%)	88 (52.1%)	0.828
	Female	83 (49.1%)	81 (47.9%)	
Area of residence	Northern West Bank**	78 (46.2%)	79 (46.7%)	0.991
	Southern West Bank***	48 (28.4%)	48 (28.4%)	
	Middle West Bank****	43 (25.4%)	42 (42.9%)	
Blood group	A	72 (42.6%)	52 (30.8%)	**0.019***
	B	27 (16%)	19 (11.2%)	
	AB	19 (11.2%)	21 (12.4%)	
	O	51 (30.2%)	77 (45.6%)	
Rhesus factor	Positive	137 (81.1%)	134 (79.3%)	0.682
	Negative	32 (18.9%)	35 (20.7%)	
Body Mass Index	<18 Underweight	6 (3.6%)	3 (1.8%)	0.739
	18-24.9 Normal	74 (43.8%)	72 (42.6%)	
	25-29.9 Overweight	55 (32.5%)	56 (33.1%)	
	>30 Obesity	34 (20.1%)	38 (22.5%)	
Marital status	Single	51 (30.2%)	63 (37.3%)	0.343
	Married	109 (64.5%)	96 (56.8%)	
	Divorced or widowed	9 (5.3%)	10 (5.9%)	
Occupation	Health Worker	12 (7.1%)	21 (12.4%)	0.132
	Non-Health employee	82 (48.5%)	87 (51.5%)	
	Unemployed	75 (4.4%)	61 (36.1%)	
Chronic Diseases	Yes	37 (21.9%)	36 (21.3%)	0.895
	No	132 (78.1%)	133 (78.7%)	
Smoking status				
Smoking	Yes	50(29.6%)	75(44.4%)	0.005
	No	119(70.4%)	94(55.6%)	
Current smoker	Yes	47(27.8%)	65(38.5%)	0.038
	No	122(72.2%)	104(61.5%)	
Age of smoking initiation	< 18 years	26(15.4%)	40(23.7%)	0.019
	≥ 18 years	24(14.2%)	35(20.7%)	
Flu Vaccination				
In past years, did you get the flu vaccination?	Yes	39(23.1%)	58(34.3%)	0.022
	No	130(76.9%)	111(65.7%)	
COVID-19 PCR test				
How many times have you done this test?	< 3 times	118(69.8%)	91(53.8%)	0.003
	≥ 3 times	51(30.2%)	78(46.2%)	
In the last Test, was there a need for a re-test?	Yes	29(17.2%)	55(32.5%)	0.001
	No	140(82.8%)	114(67.5%)	

*Significant value (P<0.05), **Northern West Bank: Nablus, Jenin, Qalqilya, Salfeet, Tubas, Tulkarem, ***Southern West Bank: Bethlehem, Hebron, ****Middle West Bank: Ramallah, Jericho, Jerusalem

Table2: Severity of COVID-19 among cases in Palestine

Variables		A	B	AB	O	P. Value
Severity of Symptoms	Mild*	36 (29.0%)	15(32.6%)	11(27.5%)	28(21.9%)	0.317
	Moderate**	15(12.1%)	3(6.5%)	3(7.5%)	14(10.9%)	
	Severe***	5(4.0%)	3(6.5%)	0(0.0%)	4(3.1%)	
	Critical***	12(9.7%)	6(13.0%)	5(12.5%)	6(4.7%)	
The duration of symptoms (days)	< 7days	21(16.9%)	8(17.4%)	4(10.0%)	16(12.5%)	0.154
	≥ 7 days	50(40.3%)	19(41.3%)	16(40.0%)	36(28.1%)	
Oxygen therapy		17 (13.7%)	9(19.6%)	5(12.5%)	9(7.0%)	0.117
Mechanical Ventilation		9(7.3%)	6(13.0%)	3(7.5%)	4(3.1%)	0.121
Duration on Mechanical Ventilation (days)	< 7days	1(0.8%)	2(4.3%)	0(0.0%)	1(0.8%)	0.108
	≥ 7 days	8(6.5%)	4(8.7%)	3(7.5%)	2(1.6%)	

On the other hand, there is no statistically significant difference between blood group A and blood group O ($P > 0.05$, AOR = 0.843, CI = 0.382-1.863), which revealed that blood group O is 0.84 times more protective than blood group A. Smoking showed no significant differences between smokers and nonsmokers ($P > 0.5$). Additionally, the study showed that not being vaccinated for seasonal flu increases the risk of COVID-19 infection by 1.97 times compared to people who get vaccinated, as shown in Table 3.

DISCUSSION

Study Participant's Characteristics and Blood Group Type with Risk of COVID-19

This section includes participants' sociodemographic factors, smoking status, BMI, occupational exposure, and seasonal flu experience. We calculated unadjusted odds ratios for each variable, which showed no significant differences except for age, which was identified as a confounder. The results showed no significant difference between cases and controls in risk of COVID-19 according to gender ($P = 0.828 > 0.05$). This result is consistent with studies from Egypt, Saudi Arabia, China, Italy, Spain, France, Germany, and Switzerland [1, 3, 11]. Few studies have examined the interaction between gender, blood type, and the severity of COVID-19, and we highlighted this aspect to explore its potential as a confounder.

Blood group

In our multivariate analysis, we compared blood group A versus other blood groups. The results showed that blood group AB (0.4%) and blood group B (0.2%) were more protective, while there was no significant difference between blood group O and blood group A (CI = 0.382-1.863, AOR = 0.843). This finding is consistent with a case-control study conducted among 105 cases and 103 controls in Wuhan, which found a significant difference between blood group and the risk of infection with COVID-19, particularly among females with blood group A [12].

Conversely, a retrospective cross-sectional study conducted in Saudi Arabia and Egypt found an insignificant difference in the distribution of blood group A between cases and controls (35% versus 29%, respectively) and no significant difference in RH factor distribution ($P = 0.191$) [3]. This discrepancy could be attributed to differences in sample size, study design, and the geographical distribution of blood groups.

Table 3: Multivariate Forward non conditional model analysis of the associated variables with COVID-19

Variables		Sig	AOR**	95% C.I.***	
				Lower	Upper
Blood group	A	.003			
	B	.002	.400	.223	.720
	AB	.004	.316	.143	.698
	O	.673	.843	.382	1.863
Smoking	Yes	Ref.****	Ref.	Ref.	Ref
	No	.493	.545	.096	3.100
Current smoker	Yes	Ref.	Ref.	Ref.	Ref
	No	.180	.379	.092	1.566
Age of smoking initiation	< 18 years	.055			
	≥ 18 years	.064	6.109	.898	41.575
Receiving flu vaccination?	Yes	Ref.	Ref.	Ref.	Ref
	No	.019	1.974	1.119	3.483
How many times have you done this test?	< 3 times	Ref.	Ref.	Ref.	Ref
	≥ 3 times	.023	.531	.308	.916
In the last Test, was there a need for a re-test to confirm the result?	Yes	Ref.	Ref.	Ref.	Ref
	No	.098	1.679	.909	3.102

All variables that were significant ($P < 0.05$) in univariate analysis were included in a multivariate model: i.e., Blood group, Smoking, current smoker, Flu vaccination **Adjusted odds ratio. ***Confidence interval. ****Ref: Reference.

The role of blood group on COVID-19 susceptibility may be related to the differential aggregation of virus glycoprotein receptors on host cell surfaces, which is influenced by ABO(H) determinants via carbohydrate-carbohydrate interactions with these receptors' glycan motifs. This interaction may interfere with virus binding and entry into target cells [13]. Other cells and tissues, such as lymphocytes, endothelial cells, platelets, gastric mucosa, and bone marrow, also express ABO(H) blood group carbohydrate structures, not just red blood cells [14]. Furthermore, blood type antigens may be present in the secretions (saliva) of approximately 80% of people (ABO secretors) [14].

Regarding the rhesus factor, our study showed no significant difference between cases and controls, similar to findings from a retrospective cohort study in Denmark, which found a significant difference between blood group and risk for COVID-19 but no significant difference between blood group or Rh factor and hospitalization outcome or death [15]. Conversely, a case-control study

conducted on 5668 COVID-19 patients and 5668 controls in Iraq demonstrated that individuals with negative RH are more susceptible to COVID-19 than those with positive RH blood type (OR = 2.38, 95% CI [2.03, 2.79], P = 0.0001) [16]. Additionally, a study in Canada concluded that the Rhesus-negative (Rh−) blood type was slightly protective against COVID-19, particularly for those with O-negative blood (aRR, 0.74 [CI, 0.66 to 0.83]; ARD, −8.2 per 1000 [CI, −10.8 to −5.3]) [17]. These differences could be attributed to variations in sample size and Rh factor distribution among countries, as the Rh-negative factor is rare in Palestine.

The following factors were assessed to determine whether they were confounding variables, but the results showed no association with the COVID-19 outcome, indicating they were not confounders in our study. No significant difference was observed between COVID-19 patients regarding their BMI, which aligns with a study conducted on 1067 patients in Southeast Asia [18]. Furthermore, no genome-wide association studies have shown that other genes linked to higher BMI are situated in the same chromosomal area as ABO, or that the ABO gene exerts regulatory control over them [18]. Therefore, it is reasonable to conclude that there is insufficient genetic evidence to link ABO to BMI. The various findings addressing the link between ABO/Rh status and BMI may be influenced by local variables that alter population phenotype or changes in sample size rather than a true genetic impact [18].

Regarding flu, our study showed no significant difference between cases and controls according to their past seasonal flu experience. However, there was a significant difference between cases and controls among those who had received a flu vaccine previously ($P < 0.05$), except for the current year. On the other hand, new evidence from a study conducted by researchers in London suggests that people who currently smoke may be less likely to get infected with coronavirus. One study found that nicotine inhibits the virus's ability to enter cells by interfering with ACE2 receptors [19].

This contrasts with a study conducted on over 2.4 million participants, which showed that current smokers were more likely to report symptoms indicative of a COVID-19 diagnosis. The adjusted odds ratios (OR) for classic symptoms were 1.14 (95% CI, 1.10 to 1.18), for more than 5 symptoms were 1.29 (95% CI, 1.26 to 1.31), and for more than 10 symptoms were 1.50 (95% CI, 1.42 to 1.58) [20]. The study suggested that smoking is linked to the overexpression of ACE2, the receptor for SARS-CoV-2 in the lungs. However, a recent meta-analysis shows mixed effects, with upregulation in epithelial cells and downregulation in alveolar type 2 cells. Internalization of ACE2 due to viral infection may result in unopposed ACE inhibitor action and high angiotensin 2 levels, contributing to endothelial damage and the coagulopathy and microthrombosis seen in severe COVID-19 patients [20, 21]. These variations in results may be due to the distribution of smokers among cases and controls, which is related to the small sample size.

Association between ABO blood group and the Severity of Symptoms

Our study results showed that the most critical symptoms were among blood group B, and the highest distribution of moderate symptoms was also among blood group B. Despite this, there is no association between ABO blood group and the severity of symptoms, which corresponds with findings from a study involving 227 COVID-19 patients. The study showed that the risk of severe COVID-19 infection did not differ significantly according to ABO blood types [22]. Conversely, a retrospective cross-sectional study conducted in Egypt and Saudi Arabia indicated a significant difference between cases and controls regarding the risk for COVID-19 but not for symptoms like fever, headache, shortness of breath, cough, bone ache, gastrointestinal symptoms, and hospitalization.

Our findings are similar to a case-control study conducted in Utah, Idaho, and Nevada, which adjusted for age, gender,

and RH factor, showing no association between blood group and the risk or severity of symptoms [23, 24]. On the other hand, there was a statistically significant difference between blood groups and the need for mechanical ventilation, myalgia, and the recovery time from COVID-19. Blood group A showed the highest percentage of individuals with low oxygen saturation, while no one in blood group B experienced low oxygen saturation. Blood group O had the highest percentage of individuals requiring mechanical ventilation, with no one in blood group A needing it [3]. These differences could be attributed to variations in sample size and blood group distribution.

Association between ABO blood group and COVID-19 outcomes

Our study revealed no significant difference between ABO blood group and COVID-19 outcomes, including intubation, need for intensive care unit admission, need for oxygen therapy, or complications. This is consistent with a study conducted in five major hospitals in Massachusetts from March to April, involving 7648 patients. The results showed that 37.5% were admitted to the hospital, 9.5% were admitted to the ICU, 8.4% were intubated, and 6.9% died. Among these patients, 34.2% had blood group A, 15.6% had blood group B, 4.7% had blood group AB, and 45.5% had blood group O. The researchers found no association between ABO blood type and COVID-19 disease severity, defined as intubation or death, with blood type O having the lowest frequency of disease positivity [25].

In contrast, a case-control study conducted among 179 patients with confirmed COVID-19 and 5200 healthy control patients in Turkey showed that blood group A was the most common among COVID-19 cases compared to the control group. It also found that patients with blood group A had longer ICU stays and higher mortality rates than others [26]. An ecological study conducted among 86 Asian, European, African, and American populations showed that blood groups A, B, and Rh- were significantly associated with COVID-19, with blood group A- having the most serious outcomes and hospitalization rates [27, 28].

Furthermore, the outcomes of this study, as well as the few other publications in this sector, indicate a wide range of data, making a conclusion about a link between blood type and COVID-19 challenging. This discrepancy in data might imply the presence of some unknown underlying factor rather than the blood group or type of antibodies present.

Study Strengths and Limitations

One of the few studies conducted in Palestine employed a case-control study design and included multiple variables, which might act as confounders. It effectively measured the risk of COVID-19 infection, the severity of symptoms, and the outcomes of infection using a valid and reliable study tool.

Regarding study limitations, there were constraints such as limited resources and scant previous research on ABO groups and COVID-19, as well as limited financial support. There was also the potential for recall bias in recalling specific symptom severities. Furthermore, misclassification of cases and controls due to false-negative or false-positive results was possible, and there were challenges in matching related to limited resources concerning potential confounders. Additionally, the study faced restrictions due to lockdown measures.

CONCLUSION

The study conducted in Palestine has identified a possible association between ABO blood group and COVID-19. Specifically, blood group “A” was found to increase the risk of COVID-19 infection. However, no association was observed regarding the severity of COVID-19 symptoms. Nevertheless, the exact mechanism and the extent of this effect varied, highlighting the need for larger and more rigorously controlled studies to investigate further.

This public health study underscores the potential influence of blood types on the transmission and infection of

individuals with the COVID-19 virus. Understanding these factors can enhance our knowledge of virus transmission dynamics and aid in developing targeted preventive measures and public health interventions to mitigate the spread of the epidemic and reduce infection rates.

Author Contributions

MK and AO: conceptualization of study and its design; AO, data collection and curation; MK and AO: data analysis and interpretation; MK and AO. Initial draft of manuscript; MK and AO: critical revision of final manuscript; MK and AO.: final approval of manuscript.

Data Availability Statement

All data collected and analyzed for this study have been deposited at the Drug

Information Library of the Department of Clinical Pharmacy, Al-Quds University. Any reader seeking to have access to these data should send a mail to the corresponding author.

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Conflict of Interest

The authors have no interest related to this study to declare.

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العلاقة بين نظام فصيلة الدم ABO وخطورة فيروس كورونا في الضفة الغربية: دراسة الحالات والشواهد

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ملخص

مقدمة: فحصت العديد من الدراسات عوامل الخطر لكوفيد-19 وكان هناك تعارض بين نتائج وتقارير الباحثين حول ما إذا كان لنوع فصيلة الدم دور في التأثير على خطر كوفيد-19. لذلك ، هدفت هذه الدراسة إلى تحديد العلاقة بين نظام فصيلة دم أبو وشدة كوفيد -19 في الضفة الغربية في فلسطين.

منهجية الدارسة: تألف تصميم دراسة الحالات والشواهد من (169 حالة و 169 عنصر تحكم) الذين اختبروا كوفيد-19 خلال شهر مارس/آذار 2021 في الضفة الغربية ، طبقية وفقا لنتائج الاختبار. تم استخدام استبيان ذاتي الإدارة لجمع البيانات وتم ملؤه عن طريق الاتصال بالمشاركين عبر أرقام هواتفهم. للتحليل ، تم إجراء التحليلات الوصفية والتحليلية. الانحدار اللوجستي الثنائي (أدخل النموذج) في التحليل متعدد المتغيرات لإثبات نسبة الأرجحية.

النتائج: فصيلة الدم ب أكثر حماية ضد كوفيد -19 من "أAB" .(AOR= 0.40, CI= 0.223-0.27) " هو أكثر حماية تجاه كوفيد -19 أكثر من"A أ .(AOR= 0.316, CI= 0.143-0.698, P < 0.5) " لا يوجد فرق ذو دلالة إحصائية بين فصيلة الدم أ وفصيلة الدم " س " و شدة الأعراض أو النتائج المركبة لكوفيد-19.

الاستنتاج: هناك ارتباط بين فصيلة دم ABO وخطر الإصابة بكوفيد-19. ومع ذلك ، اختلفت الآلية الدقيقة وشدة التأثير مما استلزم مزيدا من التحقيق من خلال دراسات أكبر وأكثر كثافة.

الكلمات الدالة: فلسطين، كوفيد-19، فصيلة الدم، شدة الاعراض.

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Knowledge and Attitude towards Vaginoplasty and Perineoplasty among Jordanian Females

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ABSTRACT

Introduction: The global increase in female genital cosmetic surgery (FGCS), including procedures like vaginoplasty and perineoplasty, has raised concerns regarding their safety and effectiveness. Therefore, this study aimed to address this gap by assessing the knowledge and attitudes of Jordanian females towards vaginoplasty and perineoplasty.

Study design: A cross-sectional online survey was conducted among 522 Jordanian females aged 18 or older.

Methods: Quantile regression models were employed to identify variables associated with females' knowledge and attitudes towards vaginoplasty and perineoplasty.

Results: The study revealed a low level of knowledge regarding vaginoplasty and perineoplasty, despite positive attitudes towards these surgeries. Significant associations were found between knowledge levels and both age and occupation. Similarly, attitudes were significantly associated with age and socioeconomic status.

Discussion: These findings underscore the need for targeted educational campaigns to enhance awareness about these procedures and their associated risks. Moreover, there should be a focus on evaluating physician-patient communication to ensure informed decision-making.

Keywords: Women's health, Vaginoplasty, Perineoplasty, FGCS, Episiotomy, Attitude, Knowledge.

INTRODUCTION

There has been a continuous increase globally in the number of females who undergo female genital cosmetic surgery (FGCS) [1]. These types of surgeries include, but are not limited to, vaginoplasty and perineoplasty [1].

Vaginoplasty is a procedure used to repair/tighten the vaginal canal or to create a vagina for transgender individuals. It is typically performed for several conditions, including congenital vaginal agenesis, pelvic trauma or tumors, as well as part of gender-affirming surgery [2, 3]. Conversely, perineoplasty is used to construct/tighten and restore function of the perineum, the muscle at the vaginal opening. Thus, it is performed on females who have loose skin around the vagina, excessive scar tissue following vaginal delivery or episiotomy, and for those who have urinary incontinence [4–6]. Vaginoplasty and perineoplasty may overlap and are often performed during a single surgery [7, 8]. Several potential complications are associated with these procedures, including dyspareunia, scarring, altered sensation,

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infection, and adhesions [9].

According to the American College of Obstetricians and Gynecologists (ACOG), vaginoplasty and perineoplasty are not considered medically indicated surgeries and thus are not endorsed by ACOG. Some surgeons claim that these procedures can increase genital sensitivity and thereby improve sexual satisfaction; however, ACOG argues against this notion due to lack of documentation on the safety and effectiveness of such procedures, as well as insufficient studies on long-term complications and patient satisfaction [9]. Therefore, females opting for these surgeries should be fully informed about the associated risks, benefits, and necessity to make an informed decision. Physicians are advised to discuss patients' reasons for seeking these surgical interventions [9].

To the best of the authors' knowledge, no studies have evaluated the knowledge and attitudes of Jordanian females toward vaginoplasty and perineoplasty procedures. Therefore, the present study aimed to assess Jordanian females' knowledge and attitudes toward these surgeries.

METHOD

Study design

This cross-sectional study was conducted online from January to May 2023. An open voluntary questionnaire was created using Google Forms and distributed through female-only Jordanian Facebook groups. The questionnaire included initial questions about participants' sex, age, and place of residence to ensure they met the inclusion criteria of being Jordanian females aged 18 or older.

Participants had access to the information and consent form online, which outlined details such as the principal investigators, co-investigators, study purpose, duration of participation, nature of participation, procedures, confidentiality measures, communication of overall results, funding details, voluntary participation rights, right to withdraw, and contact information for queries. Ethical approval for the study was granted by the research ethics committee of Al-Zaytoonah University of Jordan (ref #27/07/2022-2023).

Sampling Strategy and Sample Size

A convenience sampling technique was employed, based on a 95% significance level and a 5% margin of error, to determine the minimal required sample size, which was calculated to be 385 participants [10]

Study Instrument

The questionnaire used in this study was developed following a comprehensive literature review and consultation with an expert panel consisting of a gynecologist, a surgeon, and a clinical pharmacist. The questionnaire ensured anonymity for participants.

The first part of the questionnaire included definitions of vaginoplasty and perineoplasty procedures, explained the study's objectives, and assured respondents of their anonymity. It also gathered sociodemographic information, such as age, marital status, education level, and whether participants were studying or working in the medical field. Household monthly income was categorized as low (less than 500 Jordanian Dinars [JOD]), moderate (500-1000 JOD), and high (above 1000 JOD).

The second part assessed participants' knowledge about vaginoplasty and perineoplasty, including their complications, anesthetic requirements, and healing times.

The final part of the questionnaire consisted of eight items aimed at evaluating participants' attitudes toward vaginoplasty and perineoplasty. Positive attitude statements were rated on a 5-point Likert scale from 1 (strongly disagree) to 5 (strongly agree). These statements included beliefs that these procedures should be performed post-episiotomy, could benefit females with a loose vaginal opening, and that surgeons should obtain patient consent. Negative attitude items were also included, where scoring was reversed (1 for "strongly agree" and 5 for "strongly disagree"). These negative statements included beliefs that these procedures should be performed purely for cosmetic reasons or to satisfy a partner, as well as beliefs that all females should undergo them after childbirth or with aging, and for those with urinary problems without exception.

Survey Validity and Reliability

The questionnaire items were initially designed in English and then translated into Arabic by two linguistic professionals. The translated version was subsequently back-translated into English, and the two English versions were found to be comparable. To ensure the readability and comprehensibility of the questions, a pilot study involving 35 females was conducted. Data collected from this pilot study were excluded from the final analysis. The internal consistency of the derived latent variables (knowledge and attitude scores) was assessed using Cronbach's alpha [11], yielding a value of 0.712, which is considered acceptable [12].

Statistical analysis

Data analysis was performed using SPSS version 28.0. Frequencies and percentages were used to present categorical variables [13], while continuous variables were expressed as median (95% CI). Attitude and knowledge scores were calculated by summing scores across designated items. Cronbach's alphas were computed to evaluate the internal consistency of the latent variables. To identify variables associated with knowledge and attitude scores, two quantile regression models were constructed. Independent variables included age, education level, family income, marital status, and whether respondents were employed or studying in the medical field. The significance level was set at $p < 0.05$.

RESULTS

The study sample comprised 522 females with demographic characteristics detailed in Table 1. Of these, 188 (36%) were aged 26-35 years, whereas only 61 (11.7%) were aged 46 years and older. Regarding education, 379 (72.6%) had obtained either a bachelor's degree or a diploma, and 90 (17.2%) had pursued postgraduate education. Only 53 (10.2%) reported their highest education level as high school or elementary school. In terms of family income, 224 (42.9%) came from households with moderate financial resources, while 102 (19.5%) and 196 (37.5%) had low and high incomes, respectively. Regarding their field of work or study, 247 (52.7%) were either employed in a medical profession or studying in a medical-related field. Lastly, 306 (58.6%) of the participants were married.

Table 1 Participants' sociodemographic characteristics.

		Frequency (%)
Age	18-25	148 (28.4%)
	26-35	188 (36%)
	36-45	125 (23.9%)
	46 or older	61 (11.7%)
Level of education	High School or lower	53 (10.2%)
	Bachelor's/Diploma	379 (72.6%)
	Postgraduate	90 (17.2%)
Family income*	Low income	102 (19.5%)
	Moderate income	224 (42.9%)
	High income	196 (37.5%)
Do you work/study in a medical-related field?	No	275 (52.7%)
	Yes	247 (47.3%)
Marital status	Single	216 (41.4%)
	Married	306 (58.6%)

*Family income: Low <500 JOD, Moderate 500-1000 JOD, and High < 1000 JOD

Table 2 provides details on respondents' general knowledge of the surgical procedure. The question most frequently answered correctly was whether the procedure requires anesthesia, with 348 (66.7%) participants answering correctly. Additionally, 219 (42%) participants correctly affirmed that vaginoplasty may affect subsequent vaginal deliveries. The least correctly answered question was whether permanent vaginal discoloration is considered a possible complication, with only 99 (19%) participants answering correctly. Similarly, only 125 (23.9%) participants correctly identified stress as a potential complication following the procedure. Moreover, regarding the statement "Complete healing from vaginoplasty/perineoplasty only requires a short period of time," where "no" is the correct answer, only 109 (20.9%) participants answered correctly. The median knowledge score was 3 (ranging from 1 to 6) out of a maximum possible score of 12, and the knowledge questionnaire demonstrated acceptable internal consistency, confirmed by computing Cronbach's alpha (0.87).

Table 3 summarizes participants' attitudes towards vaginoplasty/perineoplasty. In terms of unfavorable attitudes, 183 (35.1%) participants strongly agreed or agreed that vaginoplasty should be performed for cosmetic reasons, while 128 (24.5%) believed it should be performed solely to satisfy a sexual partner. Moreover, 125 (23.9%) and 90 (17.2%) strongly agreed/agreed with the notion that all women should undergo the procedure immediately after delivery and that all elderly women should undergo the surgery, respectively. Additionally, 161 (30.8%) participants strongly agreed or agreed that all females suffering from urinary problems should undergo vaginoplasty.

In terms of favorable attitudes, 125 (23.9%) participants expressed strong disagreement or disagreement with performing the surgery after an episiotomy. Furthermore, 97 (18.6%) participants strongly disagreed or disagreed with the notion that performing vaginoplasty/perineoplasty may benefit females with a loose or stretched vaginal opening. Finally, 59 (11.3%) participants strongly disagreed or disagreed that surgeons should first ask for the patient's consent before conducting the procedure. The median score for favorable attitudes was 16 (ranging from 14 to 19) out of a possible score of 25, and the median score for non-favorable attitudes was 11 (ranging from 9 to 12) out of a maximum possible score of 15. The internal consistency of the non-favorable and favorable attitude items was acceptable, with a Cronbach's alpha of 0.81.

Quantile regression was used to assess the variables associated with knowledge and attitude scores (Tables 3 and 4). The results indicated that participants in the age group 18-25 had significantly higher knowledge and attitude scores compared to those aged 46 and older (coefficient = 2.5, 95% CI (0.759 - 4.241), p = 0.005 and coefficient = 2.5, 95% CI (0.634 - 4.366), p = 0.009, respectively). Participants who were not employed or studying in a medical field had significantly lower knowledge scores compared to their counterparts (coefficient = -1.00, 95% CI (-1.962 - -0.38), p = 0.042). Furthermore, individuals in the low and moderate family income groups demonstrated significantly lower attitude scores compared to those in the high-income group (coefficient = -1.50, 95% CI (-2.78 - -0.220), p = 0.022 and coefficient = -1.25, 95% CI (-2.235 - -0.265), p = 0.013, respectively).

DISCUSSION

The present study evaluated Jordanian females' knowledge and attitudes toward vaginoplasty and perineoplasty. In Jordan, research on female genital cosmetic surgery (FGCS), including vaginoplasty and perineoplasty, is notably scarce. This gap underscores a critical need for robust educational and awareness campaigns. The study's findings reveal a widespread lack of knowledge about these procedures among Jordanian women, despite a generally positive attitude toward them. It is evident that while there is increasing demand for FGCS, understanding and informed decision-making lag behind. The limited published research in this area signifies gaps in Jordanian women's knowledge of the prevalence, safety, efficacy, and long-term effects of

FGCS. Therefore, this study serves as a foundational step for further research and exploration in response to the growing interest and demand for such procedures.

Table 2 Females' knowledge regarding vaginoplasty/ perineoplasty complications and other related questions.

11u11d11`q1	No	I don't know	Yes
Which of the following is considered a complication from vaginoplasty/perineoplasty [Nerve damage and loss of sensation]*	70 (13.4%)	298 (57.1%)	154 (29.5%)
Which of the following is considered a complication from vaginoplasty/perineoplasty [Labial asymmetry]*	123 (23.6%)	212 (40.6%)	187 (35.8%)
Which of the following is considered a complication from vaginoplasty/perineoplasty [Permanent discoloring]*	121 (23.2%)	302 (57.9%)	**99 (19%)**
Which of the following is considered a complication from vaginoplasty/perineoplasty [Death of the tissue in the surgery area]*	74 (14.2%)	271 (51.9%)	177 (33.9%)
Which of the following is considered a complication from vaginoplasty/perineoplasty [Painful intercourse]*	70 (13.4%)	260 (49.8%)	192 (36.8%)
Which of the following is considered a complication from vaginoplasty/perineoplasty [Sexual arousal disorder]*	78 (14.9%)	297 (56.9%)	147 (28.2%)
Which of the following is considered a complication from vaginoplasty/perineoplasty [Urinary problems]*	101 (19.3%)	273 (52.3%)	148 (28.4%)
Which of the following is considered a complication from vaginoplasty/perineoplasty [Stress]*	110 (21.1%)	287 (55%)	**125 (23.9%)**
Which of the following is considered a complication from vaginoplasty/perineoplasty [May affect the next vaginal delivery]*	77 (14.8%)	226 (43.3%)	**219 (42%)**
Which of the following is considered a complication from vaginoplasty/perineoplasty [Some of the complications may require an additional surgery]*	56 (10.7%)	275 (52.7%)	191 (36.6%)
Does vaginoplasty/perineoplasty need anesthesia?*	13 (2.5%)	161 (30.8%)	**348 (66.7%)**
Complete healing from vaginoplasty/perineoplasty only requires a short period of time **	109 (20.9%)	287 (55%)	126 (24.1%)

*The correct answer is "yes".

**The correct answer is "no".

Table 3: Females' attitudes towards vaginoplasty/ perineoplasty.

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
Negative Attitudes					
I think that vaginoplasty/perineoplasty should be performed for cosmetic reasons.	**25 (4.8%)**	**158 (30.3%)**	179 (34.3%)	101 (19.3%)	59 (11.3%)
I think that vaginoplasty/perineoplasty should be performed to satisfy the sexual partner.	**19 (3.6%)**	**109 (20.9%)**	143 (27.4%)	136 (26.1%)	115 (22%)
I think that all females need to undergo vaginoplasty/perineoplasty after childbirth (without exception).	**24 (4.6%)**	**101 (19.3%)**	135 (25.9%)	163 (31.2%)	99 (19%)
I think that all old females need to undergo vaginoplasty/perineoplasty.	**12 (2.3%)**	**78 (14.9%)**	144 (27.6%)	187 (35.8%)	101 (19.3%)
I think that vaginoplasty/perineoplasty should be performed to females with urinary problems (without exception)	**26 (5%)**	**135 (25.9%)**	187 (35.8%)	119 (22.8%)	55 (10.5%)
Positive/Favorable Attitudes					
I think that vaginoplasty/perineoplasty should be performed after episiotomy.*	42 (8%)	193 (37%)	162 (31%)	**81 (15.5%)**	**44 (8.4%)**
I think that females who have loose/stretched vaginal opening may benefit from vaginoplasty/perineoplasty procedures.*	47 (9%)	251 (48.1%)	127 (24.3%)	**69 (13.2%)**	**28 (5.4%)**
I think that surgeons should take patients' consent before performing vaginoplasty/perineoplasty procedures.*	194 (37.2%)	219 (42%)	50 (9.6%)	**29 (5.6%)**	**30 (5.7%)**

Table 4. Quantile regression model for Knowledge scores (n=522).

Parameter Estimates (q=0.5)					
Parameter		**Coefficient**	***p***	**95% Confidence Interval**	
				Lower Bound	**Upper Bound**
(Intercept)		3.00	<0.01	1.34	4.66
Family income	Low income	0.00	1.00	-1.20	1.20
	Moderate income	0.00	1.00	-0.92	0.92
	High income	0	.	.	.
Marital status	Single	-0.50	0.37	-1.60	0.60
	Married	0	.	.	.
Education	High School or less	0.50	0.57	-1.24	2.24
	Bachelor's/Diploma	0.00	1.00	-1.15	1.15
	Postgraduate	0	.	.	.
Age group	18-25	2.50	<0.01	0.76	4.24
	26-35	1.00	0.16	-0.41	2.41
	36-45	0.00	1.00	-1.45	1.45
	46 and more	0	.	.	.
Work/study in a medical-related field	No	-1.00	0.04	-1.96	-0.04
	Yes	0	.	.	.

Table 5. Quantile regression model for Attitude scores (n=522).

Parameter Estimates (q=0.5)					
Parameter		**Coefficient**	***p***	**95% Confidence Interval**	
				Lower Bound	**Upper Bound**
(Intercept)		26.50	<0.01	24.72	28.28
Family income	Low income	-1.50	0.02	-2.78	-0.22
	Moderate income	-1.25	0.01	-2.23	-0.27
	High income	0	.	.	.
Marital status	Single	0.50	0.41	-0.68	1.68
	Married	0	.	.	.
Education	High School or less	-0.75	0.43	-2.61	1.11
	Bachelor's/Diploma	-0.25	0.69	-1.48	0.98
	Postgraduate	0	.	.	.
Age group	18-25	2.50	0.01	0.63	4.37
	26-35	1.50	0.05	-0.01	3.01
	36-45	1.00	0.21	-0.56	2.56
	46 and more	0	.	.	.
Work/study in a medical-related field	No	-0.25	0.63	-1.28	0.78
	Yes	0	.	.	.

Overall, this research observed a low level of knowledge about vaginoplasty and perineoplasty among Jordanian women, despite positive attitudes toward the surgery. These findings parallel those of a study conducted in Nigeria, where only 27.7% of 310 participants had any knowledge of FGCS procedures [14]. They also align with findings from an international survey on women's knowledge and attitudes toward genital appearance, which found that only 39% of women had ever read an informative article on FGCS [15].

Age was found to significantly influence participants' knowledge and attitudes toward vaginoplasty and perineoplasty. Females aged 18-25 demonstrated twice the level of knowledge about the procedures and associated risks compared to older women. Additionally, this age group exhibited more favorable and positive attitudes toward the procedures. This could be attributed to recent efforts in disseminating sexual and reproductive health information through scientific methods, particularly targeting younger demographics. Another plausible explanation is that younger women tend to be more attuned to concerns regarding the appearance of their genitalia [16].

This finding is consistent with research from Nigeria, where younger respondents were eight times more likely to be knowledgeable about FGCS procedures compared to older respondents. Moreover, reports indicate that the majority of FGCS procedures are performed on women aged 16 to 35 (Liao et al., 2010) [13]. However, these findings contrast with a study conducted in Australia, which found that sociocultural influences on seeking FGCS did not vary significantly across different age groups. In that study, females of all ages sought consultations with general practitioners (GPs) regarding concerns about genital anatomy and requests for FGCS [16].

The limited occurrence of medical discussions in the general population, primarily confined to those involved in the medical field [18], was reflected in the present study by a noticeable discrepancy in knowledge between women

who worked in the medical sector and those who did not, despite an even distribution of both groups in the sample. Women employed in the medical sector demonstrated higher levels of knowledge compared to their counterparts. In a study conducted in Saudi Arabia, most participants were obstetrics and gynecology consultants who did not routinely perform FGCS procedures. Nevertheless, their extensive knowledge enabled them to provide appropriate counseling to patients and refer them to specialists for such procedures [19]. This underscores the critical role of knowledge in patient education and decision-making regarding vaginoplasty and perineoplasty.

In contrast to earlier studies linking marital status with higher levels of knowledge [14,20], our study found no significant association between marital status and participants' knowledge or attitudes. However, it is noteworthy that married individuals still held positive attitudes toward their genital appearance and favored undergoing surgery for cosmetic reasons. This may be influenced by societal pressures and a lack of reliable information on the risks and benefits of the procedure, leading women to believe that surgery should be pursued for reasons other than medical indications, such as satisfying their sexual partner [14,20,21].

Participants from lower and moderate family income groups exhibited significantly fewer positive attitudes toward vaginoplasty procedures. This finding aligns with previous studies indicating a higher prevalence of FGCS among women from middle or upper social classes [14,22,23]. It suggests that differences in attitudes toward vaginoplasty across income groups may stem from various socioeconomic factors, with women from lower and moderate-income groups potentially prioritizing different norms or body image standards compared to those from higher income groups. While some evidence supports such a link [24], this has yet to be explored specifically in relation to vaginoplasty and perineoplasty. Alternatively, accessibility and affordability could play a role, with elective procedures potentially being less accessible to individuals with lower incomes. Therefore, women's attitudes toward the procedure might be influenced by perceptions of its cost or availability. A recent systematic review has highlighted health disparities in plastic surgery [25]. Further research should investigate the relationship between attitudes and income levels in this context.

From the standpoint of health professionals' clinical and ethical perspectives, it is argued that these surgeries should not be performed solely based on patient requests. While patient autonomy is a fundamental principle of medical ethics, the procedure may be declined if it contradicts the principle of "non-maleficence" (do no harm). Additionally, patients exercising autonomy should possess adequate knowledge regarding the procedure, including scientific data on outcomes, potential complications, and comparisons of results with non-intervention approaches [26–29].

Strengths, Limitations, and Future Research

The present study employed convenience sampling, which may have introduced bias. Participants from online female-only Facebook groups may not equally represent all demographic groups, limiting the generalizability of the findings to the broader population of Jordanian females. However, this sampling method facilitated easier access to respondents and efficient data collection, resulting in a substantial sample size of 522 participants, thereby enhancing the statistical power and reliability of the results.

The cross-sectional design used in the study provided only a snapshot of participants' knowledge and attitudes and did not allow for establishing causal relationships or tracking changes in attitudes over time.

However, the present study has several strengths that contribute to its overall validity and significance. Notably, it addresses a crucial research gap by examining Jordanian females' knowledge and attitudes toward vaginoplasty and perineoplasty. As the first study of its kind in Jordan, it provides valuable insights into how these surgical procedures are perceived by women in the country.

Another important strength is the involvement of a panel of experts during the questionnaire design phase. The inclusion of a gynecologist, a surgeon, and a clinical pharmacist ensured the validity and relevance of the questions, contributing to the quality of the data collected.

To address the identified limitations and expand the understanding of Jordanian females' knowledge and attitudes toward vaginoplasty and perineoplasty, future research should consider randomized sampling techniques to obtain a more representative sample of Jordanian females. Furthermore, incorporating in-depth qualitative methods such as interviews or focus groups could complement quantitative findings and provide deeper insights into participants' perspectives on these surgical procedures.

Educational campaigns should be evaluated to assess their effectiveness in raising awareness among Jordanian women about vaginoplasty and perineoplasty. These campaigns should focus on providing accurate information about the procedures, risks, and benefits to facilitate informed decision-making.

Investigating the relationship between attitudes toward these surgical procedures and socioeconomic factors, including income levels and cultural norms, would provide valuable insights into how these factors influence perceptions of female genital cosmetic surgery.

Future research should also explore the dynamics of physician-patient communication concerning vaginoplasty and perineoplasty. This investigation can shed light on how well patients are informed about the risks, benefits, and alternative treatment options.

Moreover, assessing the psychosocial impact of vaginoplasty and perineoplasty on women who have undergone these procedures can provide valuable information about their experiences, body image, and quality of life post-surgery.

Lastly, conducting comparative studies between different cultural contexts and countries can offer a broader understanding of the social and cultural factors influencing attitudes toward female genital cosmetic surgery.

CONCLUSIONS

In this study, women demonstrated positive attitudes towards vaginoplasty/perineoplasty, despite overall low levels of knowledge about the procedures. Factors influencing women's knowledge included age and occupation, while age and income level were associated with their attitudes towards these surgeries. To address this knowledge gap and promote informed decision-making, several strategies are recommended.

Firstly, developing comprehensive educational programs covering the medical, psychological, and social aspects of vaginoplasty/perineoplasty—such as indications, procedures, potential complications, and realistic outcomes—would be beneficial. Secondly, enhancing healthcare communication is crucial. This involves training healthcare professionals to initiate sensitive discussions about these procedures and ensuring that women receive accurate and relevant information to guide their decisions.

Creating informative materials (e.g., leaflets, videos) that disseminate accurate knowledge is essential for patient education. These materials should be accessible, culturally sensitive, and suitable for varying literacy levels.

Lastly, further research is needed to understand the long-term effects of vaginoplasty/perineoplasty and the psychological and social motivations behind these procedures. Implementing these strategies would improve understanding and attitudes towards vaginoplasty and perineoplasty, empowering women to navigate their choices more effectively and confidently.

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None.

Conflict of Interest

The authors have no conflicts of interest to disclose.

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المعرفة والاتجاه نحو عمليات تجميل المهبل وتجميل العجان لدى الإناث الأردنيات

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ملخص

مقدمة: أدى الارتفاع العالمي في الجراحة التجميلية للأعضاء التناسلية الأنثوية (FGCS)، مثل عمليات تجميل المهبل والعجان، إلى إثارة المخاوف بشأن سلامتها وفعاليتها. لذلك، هدفت الدراسة الحالية إلى معالجة هذه الفجوة من خلال تقييم معرفة وموقف الإناث الأردنيات تجاه عمليات تجميل المهبل وتجميل العجان.

تصميم الدراسة: تم إجراء دراسة مسح مقطعي عبر الإنترنت على 522 أنثى أردنية تبلغ أعمارهن 18 عامًا أو أكثر.

المنهجية: تم بناء نماذج الانحدار الكمي لتحديد المتغيرات المرتبطة بمعرفة الإناث وموقفهن تجاه تجميل المهبل والعجان.

النتائج: لوحظ انخفاض مستوى المعرفة فيما يتعلق بتجميل المهبل/تجميل العجان، على الرغم من المواقف الإيجابية تجاه العمليات الجراحية. وكانت هناك علاقة ذات دلالة إحصائية بين مستوى المعرفة وكل من العمر والمهنة. وبالمثل، أظهر مستوى الاتجاه ارتباطًا كبيرًا بالعمر والحالة الاجتماعية والاقتصادية.

المناقشة: تسلط النتائج الضوء على أهمية الحملات التثقيفية المستهدفة لتحسين الوعي بهذه الإجراءات والمخاطر المرتبطة بها. يجب تقييم التواصل بين الطبيب والمريض لضمان اتخاذ قرار مستنير.

الكلمات الدالة: صحة المرأة، تجميل المهبل، تجميل العجان، الجراحة التجميلية للأعضاء التناسلية الأنثوية، قَصّ العجان، الموقف، المعرفة.

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Behavioral Interventions with and without Pharmacological Treatment: A Comparative Study at An Autistic Center in Jordan

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ABSTRACT

Autism spectrum disorder (ASD) is a complex and highly variable neurodevelopmental disorder. This manuscript describes an observational study aimed at evaluating the impact of behavioral and pharmacological treatments on autistic patients at a Jordanian Autism Institution. Patients were evaluated every three months to assess their progress in behavioral treatment using the ABLLS (Assessment of Basic Language and Learning Skills) method, which evaluates 25 skills based on a child's performance. The researcher collected all previous assessments and observed at least two evaluation periods. Males comprised 90.38% of the study population. Among psychiatric conditions, hyperactivity (36.54%) and irritability (30.77%) showed the highest prevalence. The first interval served as the baseline. Results from the second and third intervals showed slight differences but were not statistically significant. In contrast, the fourth interval demonstrated a statistically significant difference ($P < 0.0001$). In conclusion, combining pharmacological and behavioral treatments appears more beneficial than behavioral therapy alone, although this benefit may take at least a year to manifest effectively.

Keywords: ASD; Risperidone; ABA; ABLLS.

INTRODUCTION

Autism spectrum disorder (ASD) is a complex and highly variable neurodevelopmental condition [1]; worldwide prevalence is 1 in 100 according to the World Health Organization [2, 3]. Lack of social interaction, impaired communication, and restricted and repetitive behaviors are common in all patients [4]. Scientists from the Centers for Disease Control and Prevention (CDC) have reported that in the United States, the proportion of children aged 3–17 years with a developmental disability increased from 16.2% during (2009–2011) to 17.8% in (2015–2017) [5, 6]. This rise may partly stem from improved detection tools, diagnostics, and increased awareness among parents and healthcare providers [5, 7, 8].

To date, there have been no studies in Jordan estimating the prevalence of ASD. However, based on studies from other Middle Eastern countries, it is estimated to range between 0.63 and 1.76 cases per 1000 people [9]. Although official data on the prevalence of autism in Jordan is lacking due to limited regional research, experts estimate that there are approximately 8000 individuals with autism in the country [10]. Males are more affected than females, with a long-standing ratio of 4 males to 1 female [11], although recent reports suggest this ratio is now 3 males to 1 female [12].

ASD is a broad category that has replaced previous terms such as autistic disorder, Asperger's syndrome,

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childhood disintegrative disorder, and unspecified pervasive developmental disorder [13]. Symptoms of ASD may appear before the age of two years but are typically more noticeable between ages two and three [14]. The age of diagnosis has decreased in recent years, partly due to improved screening methods, with an average age of diagnosis around 60 months [15]. Children with ASD face varying degrees of developmental challenges in behavior, communication, and socialization [16, 17]. While fundamental areas may improve with age, certain symptom subdomains such as social smiling, emotional expression, and restricted interests may remain unchanged, contributing to the poor outcomes associated with adulthood [18-20].

Despite its growing prevalence, the pathophysiology of ASD remains poorly understood, which may be related to challenges in developing appropriate animal models and understanding the complexities of brain function [21]. Numerous studies indicate that significant genetic and environmental factors contribute to the increasing incidence of ASD in children [22, 23]. ASD is often recognized as a multifactorial condition caused by both hereditary and non-genetic risk factors [24]. A growing body of literature reports that genetic factors play a role in ASD development, with siblings born into ASD families being more likely to acquire the condition [25]. Regarding environmental factors, several variables are considered ASD risk factors, including advanced parental age, maternal smoking, assisted reproductive technologies, nutritional issues, maternal infections and disorders, environmental pollutants, toxic exposures, and medications [26, 27].

The primary treatment for children with ASD is early intervention with behavioral, occupational, and speech therapy to promote healthy development and sociability [28]. No pharmacological treatment has demonstrated efficacy in clinical trials or received approval from regulatory authorities for treating core ASD signs and symptoms [29]. However, pharmacological therapies are sometimes prescribed adjunctively to address co-occurring behavioral issues and support children's development and social functioning. They are also used to manage related comorbidities such as hyperactivity, sleep difficulties, and gastrointestinal issues, which may not improve with rehabilitation therapy alone but do not target core impairments [16, 17, 30-32].

Interventions based on Applied Behavior Analysis (ABA) principles are commonly used with children diagnosed with ASD and have been extensively studied [33]. ABA employs behavioral principles to identify environmental factors influencing socially significant behaviors and to design individualized interventions [34].

The current study assessed the effects of combining pharmacological and behavioral management for autistic patients at the Autism Academy of Jordan, a private center in Jordan. We chose to focus on a single center's patient population to minimize variability in diagnosis and treatment approaches across different medical centers.

METHODOLOGY

Study Design and Setting

This retrospective-prospective observational study [35] evaluated the effects of pharmacological treatment as an adjunct to behavioral therapy in managing autism among patients at a private center in Jordan. The study included all patients at the center at the beginning of the research, following IRB approval. The researcher retrieved previous assessment results from patients' records and personally observed at least two assessments during the study period (December 2016 to June 2017). This center was selected due to its large patient population and consistent use of the ABLLS evaluation tool.

Study Population

Medical records of 54 male and female patients, both inpatient and outpatient, were included in the study. Records of 2 patients were excluded due to incomplete assessments. Inclusion criteria required patients to be diagnosed

according to DSM-5 criteria by an autism specialist at the center, of any gender and age, and residing both internally and externally while exclusively treated at the Autism Academy of Jordan. All patients received behavioral therapy using the Applied Behavior Analysis (ABA) system for a minimum of 30 hours per week. To be included, patients needed to have more than one assessment period, with the first assessment serving as the baseline. The intervention options included behavioral therapy alone or combined with pharmacological treatment. The intervention spanned four intervals, each lasting three months, with assessments conducted at the end of each interval. The first interval served as the baseline assessment. Patients were followed up every three months to evaluate improvement using the ABLLS system.

The researcher (AA) was familiar with the patients' identities as she collected data from their records and observed their behavioral therapy sessions, where their names were used.

Data Collection

Social, demographic, and clinical data of patients were collected from their medical records to assess potential associations with disease prevalence or therapy progress. Demographic data included: age, gender, birth order among siblings, mode of delivery, nationality, family history of psychological or neurological disorders, housing type (internal/external), and parents' request for therapy. Clinical data encompassed EEG findings, epilepsy diagnosis, age at diagnosis, and age at enrollment in the center. It also included comorbid psychiatric conditions and symptoms such as Attention-Deficit/Hyperactivity Disorder (ADHD), bipolar disorder, tantrums, irritability, hyperactive symptoms, insomnia, aggression, and self-harm, along with details of medications used, including name, dose, and indication.

Assessment of therapeutic interventions: Behavioral intervention utilized the Applied Behavior Analysis (ABA) system, which applies behaviorist principles directly to improve human behavior. Each patient received ABA for at least 30 hours per week (6 hours per day, 5 days a week). No additional home-based or external behavioral interventions were provided. Periodic assessments using the ABLLS system were conducted every 3 months to evaluate the efficacy of the ABA system. Effectiveness was measured by overall progress across all skills. The researcher attended these evaluation sessions, and medication adherence was ensured by observing medication intake in front of the therapist.

Medication doses were gradually adjusted to balance efficacy and minimize side effects, tailored to each patient's body weight and severity of ASD. The maximum dose of risperidone used was 2 mg/day.

The ABLLS system was employed by the center to monitor progress in behavioral therapy with or without pharmacological interventions. ABLLS is an educational tool used to assess strengths and weaknesses in 25 areas of basic functional communication skills in patients with developmental delays or disabilities. Each of the 25 skills is ranked in ascending order based on complexity across multiple subdivisions. The following skill areas were measured: A-cooperation and reinforce effectiveness (A1-A19), B- visual performance (B1-B27), C-receptive language (C1-C57), D-limitation (D1-D27), E-vocal limitation (E1-E20), F-requests (F1-F29), G-labeling (G1-G47), H-intra verbal (H1-H47), I-spontaneous vocalizations (I1-I9), J-syntax and grammar (J1-J20), K-play and leisure (K1-K15), L-social interaction (L1-L34), M-group instruction (M1-M12), P-generalized responding (PA-P6), Q-reading (Q1-Q17), R-math (R1-R29), S-writing (S1-S10), T-spelling (T1-T7), U-dressing (U1-U15), V-eating (V1-V10), W-grooming, X-toileting (X1-X10), Y-gross motor (Y1-Y30), Z fine motor (Z1-Z28). Each assessment was scored and converted into a percentage of achievement. Advantages of ABLLS include providing a quick overview to parents and therapists about the patient's skill levels, and it can

be administered with minimal understanding of ABA principles. However, its lack of full standardization is considered a drawback of the ABLLS system.

Statistical Analysis

Descriptive analyses were used to summarize the data of the total sample. Continuous data were expressed as means ± standard error of the mean, while categorical data were presented as numbers and percentages. Categorical variables were analyzed using the Chi-square (χ^2) test or Fisher's exact test as appropriate.

Each score in every assessment was transformed into a percentage achieved. An overall percentage was calculated by summing percentages from all letters A to Z. A t-test was used to assess significant differences in overall percentage efficacy between the two treatment modes (behavioral vs. pharmacological plus behavioral). Additionally, two-way ANOVA was used to evaluate significant differences in overall percentage between the two intervention modalities across different assessment intervals. Statistical analyses were performed using SAS 9.2 (Cary, NC, USA). All significant differences were considered at $\alpha \leq 0.05$.

Ethical statements

Approval for this study was obtained from the Institutional Review Board (IRB) of the Ethical Committees at Jordan University of Science and Technology, Deanship of Research (Grant Number 2016/462).

Consent:

Patients at the center were unable to provide consent. Consent was obtained from their legal guardians (parents) upon admission to the center. The center provided access to records with legal confirmation.

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RESULTS

Demographics:

Data were collected from patient records at the Autism Academy of Jordan, with fifty-two participants enrolled in the study. As shown in Table 1, the majority of participants (41, 78.85%) were aged between 4 to 10 years, and 47 (90.38%) were male. Most participants ranked second among siblings in their families. Eighteen patients (34.62%), fifteen patients (28.85%), eight patients (15.38%), and seven patients (13.46%) were classified as first, third, and fourth in birth order, respectively. The mode of delivery for most patients (33, 63.46%) was vaginal birth, with the remainder (19, 36.54%) delivered via cesarean section. The majority of patients were housed internally (44, 84.62%) and were of Libyan nationality (33, 63.46%). Further details are included in Table 1.

Parents-requested behavioral modifications:

The therapist considers parents' requests to focus on and guide them to special programs designed according to their wishes and applied within the home. Specifically, 45 parents (86.54%) requested behavior modification, whereas only 4 parents (7.69%) requested speech skill modification. Further details are illustrated in Table 2.

ASD characteristics

Eight patients (15.38%) had confirmed epilepsy diagnosis; none of them had recorded changes in EEG, while six patients (11.53%) were not diagnosed with epilepsy but had sharp waves in EEG record. Details in Table 3.

Among psychiatric related conditions[36], hyperactivity symptoms (19 patients, 36.54%) followed by irritability (16, 30.77%) had the highest prevalence. Further details are shown in Table 4.

Table 1: Social and demographic Data

Social and demographic data		Number	Percentage (%)
Age	4-10 years	41	78.85
	11-17 year	9	17.31
	18-24 year	2	3.85
Gender	Male	47	90.38
	Female	5	9.62
Rank among siblings	1st	15	28.85
	2nd	18	34.62
	3rd	8	15.38
	4th	7	13.46
	5th, 6th, 7th, 8th	4	7.69
Mode of delivery	Vaginal	33	63.46
	Cesarean Section	19	36.54
Internal/External Housing	Internal Housing	44	84.62
	External Housing	8	15.38
Nationality	Libyan	33	63.46
	Saudi	14	26.92
	Jordanian	2	3.85
	Kuwaiti	2	3.85
	Iraqi	1	1.92

Table 2: Parents' request of therapist

Parent request	Frequency	Percentage%
Behavior Modification	45	86.54
Speech	4	7.69
Independence, toilet training	2	3.84
Learn to walk	1	1.92

Table 3: Epilepsy diagnoses and EEG changes

Condition	Confirmed epilepsy N (%)	No epilepsy diagnosis N (%)	Total
No changes in EEG	8 (15.38%)	38 (73.08%)	46 (88.46%)
Sharp waves in EEG	0 (0%)	6 (11.53%)	6 (11.53%)
Total	8 (15.38%)	44 (84.61%)	52 (100%)

Table 4: Frequency and percentage of psychiatric conditions among patients.

Condition	N (%)
Hyperactive symptoms	19 (36.54)
Irritability	16 (30.77)
Self-Harming	4 (7.69)
ADHD	4 (7.69)
Insomnia	2 (3.85)
Aggression	2 (3.85)
Bipolar disease	1 (1.92)
Tantrum	0 (0)

Pharmacological treatment and indications: Various psychiatric and neurological conditions associated with ASD include attention deficit hyperactivity disorder (ADHD), schizophrenia, bipolar affective disorder, eating behavior problems, and self-harming, which warranted medication use [37]. As shown in Table 5, corresponding medications, names, and prescribed doses were detailed alongside concurrent conditions. Risperidone was the most frequently prescribed medication during this study.

Therapeutic assessments

The ABLLS system was utilized by the center to monitor progress in behavioral therapy with or without pharmacological interventions. For each assessment, the calculated score was converted into a percentage achieved. An overall percentage was derived by summing the percentages for all letters from A to Z.

There were four assessment intervals, with the first interval serving as the baseline. No significant difference was observed between the two groups—behavioral therapy with pharmacological treatment and behavioral therapy alone (p = 0.3103). Similarly, no significant differences were found in the second and third intervals (p = 0.0509, p = 0.0691). However, interval four showed a statistically significant difference between the two groups ($p < 0.0001$). Therefore, approximately twelve months were needed to observe a significant improvement when medications were added to behavioral therapy. Details are provided in Table 6.

DISCUSSION

Multimodal therapy that is holistic in nature is critical for managing ASD. Our study is considered unique as it is the first of its kind. To the best of our knowledge, there is a lack of studies, especially in the Middle East region, comparing the effects of medication on behavioral interventions. Therefore, the outcomes of this study could lay the groundwork for future similar studies. One significant finding from the data is that medication combined with behavioral therapy was more effective than behavioral therapy alone. Additionally, our study found a higher prevalence of ASD in males compared to females, consistent with existing systematic reviews indicating a higher prevalence among males [38]. However, this study did not aim to estimate the male-to-female ratio or study prevalence.

Our study contrasts with previous research suggesting a significantly higher risk of ASD recurrence in siblings than previously estimated. An earlier multinational study reported an 8.4-fold increased risk of ASD after an older sibling's diagnosis [25]. However, other research indicated that 18.7% of children with at least one older sibling with ASD developed the condition [39]. Interestingly, contrary to previous assumptions, our study found that the prevalence was highest among second-born offspring (18, 34.62%), followed by

firstborn offspring (15, 28.85%), with the lowest rate observed among eighth-born offspring (1, 1.92%). To our knowledge, such a ranking among siblings has not been previously studied, possibly due to cultural perceptions in Middle Eastern countries where families may have larger numbers of children, extending to eighth or ninth births.

Table 5: concurrent medical conditions and medications used for each.

Condition	Medication (Doses)
ADHD	Omega 3 (5 ml) Atomoxetine (18 mg) Quetiapine (50 mg) Citicoline (600 mg)
Aggression	Risperidone (0.5, 0.75 mg)
Hyperactive symptom	Risperidone (0.25, .5, .75, 1, 1.5, 2 mg) Atomoxetine (10, 60 mg) Quetiapine (25 mg) Multivitamin Omega 3(5ml)
Insomnia	Melatonin (5 mg)
Self-Harming	Risperidone (0.5, 0.75, 2 mg) Olanzapine (7.5 mg)
Bipolar disease	Quetiapine (25 mg)
Irritability	Risperidone (0.5, .75, 1, 1.5, 2 mg) Olanzapine (7.5 mg) Quetiapine (25 mg) Multivitamin
Tantrum	Not seen
Speech	Valproic acid (400 mg)
Epilepsy	Carbamazepine (200, 280 mg) Levetiracetam (400, 500, 1500 mg) Valproic acid (400, 600, 900 mg) Lamotrigine (100 mg) Phenytoin (300 mg) Topiramate (50 mg) Oxcarbazepine (900 mg)
Sharp Waves in EEG	Levetiracetam (400, 600, 30000 mg) Carbamazepine (400, 800 mg) Valproic acid (600mg)
Unknown reason	Olanzapine (10 mg) Escitalopram (10 mg) Propranolol (10 mg)

Table 6: Assessment results per each of four intervals

	Number	Mean	Minimum	Maximum	P value
Interval One					
Non-Medicated	26	2.1440	0.7178	6.0474	0.3103
Medicated	25	2.7361	0.7178	11.1988	
Interval Two					
Non-Medicated	26	4.0396	1.0310	9.4099	0.0509
Medicated	26	6.0774	1.3267	18.4618	
Interval Three					
Non-Medicated	23	4.3610	1.2170	10.0612	0.0691
Medicated	23	6.4499	1.4929	18.8911	
Interval Four					
Non-Medicated	16	4.6959	2.0331	10.3461	< **0.0001**
Medicated	17	8.1218	2.3912	19.4966	

Patients were more frequently delivered via vaginal delivery, with 33 (63.46%) births, compared to 19 (36.54%) via cesarean section, despite a multinational cohort study suggesting a modest increase in ASD risk associated with cesarean delivery compared to vaginal delivery [40]. This study was conducted at a single center with a small number of patients, so generalizations about risk factors cannot be made.

The therapist considered parents' requests to tailor behavioral treatments accordingly. The highest request was for general behavior modification, with 45 (86.54%) parents, while 4 (7.69%) parents specifically requested speech-related interventions. Parents requesting behavior modification strategies such as self-management and joint attachment were trained to implement these techniques at home. Parents seeking speech-related interventions were taught narrative-based strategies to describe activities to their child and label objects.

Since the Food and Drug Administration (FDA) has not approved medications for treating core symptoms of ASD, behavioral symptoms such as hyperactivity and irritability are primarily managed. However, aripiprazole and risperidone have been FDA-approved to manage irritability in ASD patients [41] [37]. A recent systematic review highlighted significant improvements in autism-related symptoms with risperidone compared to placebo, reporting a 43% improvement in irritability on average [42]. At this center, risperidone was the most frequently prescribed and administered medication.

Divalproex sodium has demonstrated significant efficacy in controlling aggression and irritability in ASD patients in a randomized controlled trial [43]. In this study, valproic acid, carbamazepine, and levetiracetam were used for epileptiform EEG patterns, even in cases where epilepsy diagnosis was not confirmed. Sharp waves were observed concurrently with aggression and irritability, and both antipsychotic and antiepileptic drugs were employed for management.

Therapy management assessments were conducted every three months. In the first three months, there was no significant difference between the two intervention groups (behavioral therapy with pharmacological treatment versus behavioral therapy alone). During the second and third intervals, there was an increase in mean and maximum values, indicating improvement in learned skills among those receiving medication in addition to behavioral therapy. However, this difference was not statistically significant when comparing the combined pharmacological and behavioral therapy to behavioral therapy alone. A noticeable and significant difference was observed only after the fourth interval. This suggests that it takes a considerable amount of time to observe the effects of medication when combined with behavioral

therapy compared to behavioral therapy alone.

A study supporting our findings indicated that combining behavioral therapy with antipsychotic medication was the most effective method for controlling aggressive symptoms in ASD patients, albeit requiring at least 30 sessions to achieve prominent results [44].

CONCLUSION

In conclusion, our research emphasizes the importance of combining pharmacological and behavioral therapies, which proves more effective than behavioral therapy alone. However, achieving this favorable outcome typically requires at least 12 months. Both behavioral and pharmacological therapies necessitate time to achieve efficacy. Symptoms such as aggressiveness, irritability, hyperactivity, or ADHD must be managed consistently for at least 12 months to optimize the effectiveness of behavioral therapy.

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Conflict of Interest: all authors declare they have no conflict of interest.

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تدخلات سلوكية مع وبدون علاج دوائي: دراسة مقارنة في مركز للتوحد في الأردن

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ملخص

اضطراب الطيف التوحدي هو اضطراب عصبي معقد ومتغير للغاية. يصف هذا المخطوط دراسة مراقبة هدفها تقييم تأثير العلاج السلوكي والدوائي على المرضى الذين يعانون من التوحد في مركز التوحد في الأردن. تم تقييم المرضى كل ثلاثة أشهر لتحديد تقدمهم في العلاج السلوكي باستخدام طريقة (ABLLS) . قام الباحث بجمع جميع التقييمات السابقة للمرضى المتواجدين في المركز، وشهد على ما لا يقل عن تقييمين في كل فترة. النتائج : يشكل الذكور نسبة (90.38%).، بين الحالات النفسية، يظهر اضطراب الفرط النشاطي 19 (36.54%)، تليه التهيج 16 (30.77%) بأعلى انتشار. كانت الفترة الأولى هي الفترة الأساسية. في الفترتين الثانية والثالثة، تظهر النتائج فرقاً طفيفاً ولكنه غير ذلك بشكل ذي دلالة إحصائية. من ناحية أخرى، أظهرت الفترة الرابعة فارقاً ذا دلالة إحصائية، .P <0.0001. في الختام، يعتبر الجمع بين العلاج الدوائي والعلاج السلوكي أكثر فعالية من العلاج السلوكي بمفرده، على الرغم من أن هذه الفائدة تتطلب على الأقل سنة لتكون فعالة.

الكلمات الدالة: اضطراب طيف التوحد، ريسبيريدون، تحليل السلوك التطبيقي، تقييم اللغة الأساسية ومهارات التعلم.

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Community Pharmacists' Perspectives on Offering Discounted Prices for Prescription Drugs in Jordan

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ABSTRACT

Background: In Jordan, medicines are priced by the Jordan Food and Drug Administration (JFDA), which mandates pharmacists to sell pharmaceutical products without any discounts or rebates. However, discounting drug prices in community pharmacies is commonplace. The present study aims to understand the motives and perceptions of pharmacists regarding drug price discounts in community pharmacies in Jordan.

Methods: Qualitative interview sessions were conducted with a convenient sample of 20 community pharmacists. A total of 25 participants were interviewed, anonymized, and audio recorded. To ensure maximum comfort, respondents were given the option to choose the interview location or participate through audio-visual communication tools. The sessions followed a pre-designed interview guide focusing on discounts offered on prescription drugs in community pharmacies, pharmacists' experiences, and their attitudes toward these practices. Interview recordings were transcribed and analyzed thematically.

Results: Three main themes emerged: patient-related factors, pharmacist-related factors, and rules and regulations-related factors, each with several subthemes. This study identified several barriers that hinder pharmacists' adherence to medicine pricing policies in community pharmacies in Jordan, including psychosocial and economic factors, customer attraction and profit increase strategies, sales tactics, market share expansion, avoidance of medicine accumulation and expiration, and issues related to regulations and law enforcement.

Conclusion: The findings demonstrate that adherence to medicine pricing policies in community pharmacies in Jordan varies depending on different factors. Pharmacists may exhibit weak adherence under specific circumstances while demonstrating stronger adherence under others. This study provides insights that could inform the revision of regulations and laws governing pharmacy practices and adherence to pricing policies.

Keywords: Pricing, Discounting of medicines, Community pharmacies, Jordan, JFDA

INTRODUCTION

Jordan is a lower middle-income developing country in the Middle East (1). Its gross domestic product (GDP) is approximately $45 billion, with a per capita GDP of $4,204.5. The total health expenditure in Jordan is almost 8% of the GDP, and pharmaceutical expenditure accounts for 2% of the GDP, comprising 27% of the total health expenditure, estimated to be more than $334 million (2). Compared to estimates from other countries classified by the World Health Organization (WHO) as Eastern Mediterranean Region

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(EMR) countries, expenditures in Jordan are considered high (3). The increasing burden of health issues and the necessity for affordable drugs place significant pressure on individuals seeking healthcare and medications. Many patients attempt to negotiate treatment and medication prices to alleviate economic burdens (4).

The WHO constitution asserts that all people have the right to quality healthcare, and medicines play a crucial role in achieving this. Access to a variety of medications reduces mortality and morbidity, assuming affordability. This right is universal and enshrined in various healthcare policies and systems globally (5). Access is influenced by factors such as availability, affordability, and drug pricing. Negative factors, such as limited supply or high prices, render drugs inaccessible to segments of the population and can lead to adverse health outcomes (6).

In evaluating costs and access, Jordan's healthcare system financing consists of three sources: public funding (50% in 2015), including the Ministry of Health (MoH), Royal Medical Services (RMS), Civil Health Insurance Program, the Royal Court, and others; household spending (42%), which refers to insurance, direct (out-of-pocket) expenditures on private healthcare services and medications; and donor contributions (8%), encompassing medical services provided by non-governmental agencies (NGOs) (7).

Despite approximately half of the population being covered by MoH and RMS, higher expenditures (58%) occur in the private sector, indicating that even those eligible for public sector services choose private services instead (8).

Medicines in Jordan are priced by the Jordan Food and Drug Administration (JFDA), and regulations mandate pharmacists to sell pharmaceutical products to the public without discounts or rebates (9). The Jordan Pharmacist Association (JPA) is the official union for Jordanian pharmacists, collaborating with various healthcare bodies in the Kingdom such as the JFDA, RMS, and MoH. Every registered pharmacist in Jordan is a member of the JPA by law, totaling approximately 30,000 members in 2023. The JPA plays a crucial role in raising awareness among pharmacy professionals to ensure medicines are sold at regulated prices and takes a firm stance against unauthorized discounts in retail pharmacy sales. Additionally, the JPA is the sole authorized body for printing price labels on medicines sold in the Jordanian market (10).

Joint efforts by the JFDA and JPA are demonstrated through various awareness campaigns, mystery shopping programs, and the enforcement of fines and penalties (9). Despite these efforts, discounts and rebates remain common practice in community pharmacies in Jordan (11). The Jordanian public continues to demand discounts and is accustomed to negotiating medication prices. The average profit margin for pharmacists selling pharmaceutical products in Jordan is 20% (12). This study aims to investigate the reasons behind why some Jordanian pharmacists choose to offer discounts on pharmaceutical products.

Literature indicates that pharmacists play an important primary care role due to their accessibility (13). Evidence from Jordan suggests that patients often visit pharmacies to save money by purchasing prescription medications directly, without a prescription from a physician (14). We hypothesize that one reason pharmacists offer discounts is to enhance the marketability of their pharmacies, within the context of general poverty and high unemployment rates in the population (15). It is noteworthy that institutional payers for pharmaceuticals are increasingly negotiating discounts off the official listing price of pharmaceuticals purchased in the community setting worldwide (16). Discounts and rebates granted by pharmacists to the public are documented in the literature (17). However, these discounts and rebates may negatively impact the net profit of community pharmacies and potentially lead to closures (18).

This study is particularly important as well as innovative in that it aims to understand the motives behind drug price discounts in community pharmacies in Jordan and generate a

hypothesis about the discounting practice in community pharmacies that can later be tested on a larger scale.

METHODOLOGY

Study Design and Setting

In this qualitative, inductive approach study, in-depth interviews were conducted with a selected group of pharmacists to understand the motives behind offering price discounts on medications sold in community pharmacies. An inductive approach was employed for coding the data. The study was conducted over a 1-month period starting in February 2021.

The study protocol was approved by the Institutional Review Board (IRB) at Jordan University of Science and Technology (reference number: 748/2020). Written informed consent was obtained from all participants after explaining the study objectives. Participants were assured that their participation was for scientific research purposes and would involve no anticipated harmful effects. Confidentiality of their responses was maintained, and participants were informed of their voluntary participation right to withdraw at any time. The study included three sections of interview plans, detailed in the Study Interview Guide.

Study Population, and Sampling Procedure

The study sample comprised 30 pharmacists in Jordan. A convenient sampling method was used, initially identifying pharmacies through internet searches. Pharmacists were contacted using collected phone numbers. Inclusion and exclusion criteria were uniform for both objectives of the study.

Inclusion criteria:

1. Jordanian pharmacist.
2. Provided written or verbally informed consent to participate in the study.
3. Gave sufficient information for taking part in this study.

Exclusion criteria:

Pharmacists who did not consent to participate or refused audio recording were excluded.

Every participant in the study received a consent form outlining the voluntary nature of participation. One-on-one phone interviews were conducted, with the option of face-to-face interviews if preferred. Participants were informed that interviews would be recorded. To maximize comfort, interviews could be conducted at a location of their choice or via audio-visual communication tools. Each session lasted between half an hour to an hour. After conducting 25 interviews, data collection achieved saturation based on the principle of theoretical sufficiency (19).

Interviews with pharmacists were conducted privately in community pharmacies, using Jordanian Arabic for discretion and confidentiality. Participants were assured that interviews would be anonymized and recorded. A structured interview guide focused on understanding medication price discounts in community pharmacies and barriers to adherence to pricing policies. Researchers took notes during sessions to capture key points. Interviews began with questions about the pharmacist's sociodemographic profile, followed by discussions such as: "What are the advantages of offering discounts on drug prices to the public?", "What are the disadvantages?", and "What obstacles do pharmacists face in offering drug price discounts?" Subsequent questions and discussions followed the structured interview guide (see Appendix 1). Participants were encouraged to express their views through closed-ended statements, with follow-up probing questions like "What do you mean by that?" or "Could you elaborate, please?" All interviews were conducted by the same researcher to ensure consistency of understanding.

Data collection involved assigning each participant a number to maintain anonymity, with access restricted to the authors. Although 30 participants were planned, only 25 responded and were enrolled in the study.

Study Interview-guide

The interview-guide assesseas and evaluates barriers to adherence to medication pricing policies amongst community pharmacists. An interview-guide was developed after an extensive literature review done by 4 experts in pharmacy practice and health services management (14)(17)(20)(21)(22)(23)(24)(25). It consisted of two parts:

The interview guide assesses and evaluates barriers to adherence to medication pricing policies among community pharmacists. The interview guide was developed following an extensive literature review conducted by four experts in pharmacy practice and health services management (14, 17, 20-25). It consists of two parts:

First Part: Includes socio-demographic information about the participant such as gender, years of experience, pharmacy location, and position.

Second Part: Covers information on the perceived advantages and disadvantages of offering discounts on medicine prices to the public, the motives behind pharmacists providing such discounts, reasons for public demand for discounts, perceived difficulties faced by pharmacists in providing discounts, and the effectiveness of laws and regulations preventing discounts.

Analysis

Interviews were digitally recorded and transcribed verbatim. Two certified translators performed forward and backward translations, which were reviewed by a Pharmacy Practice consultant (26, 27). Results were imported into QSR International's NVivo 11 Software® (28). All audio recordings and interviewer notes were also imported into NVivo for assessment and analysis. Thematic analysis was conducted by the authors based on transcriptions. Direct quotations were lightly edited to remove non-essential content (repetitions, stutters, etc.) and corrected for grammar. Ellipses were used to indicate removal of such content. Square brackets were used in quotations to clarify omitted words or replace sensitive information like names.

Descriptive statistical analysis of the collected data was performed using Excel software®. Descriptive statistics were used to summarize socio-demographic variables, with mean and standard deviation for continuous variables, and frequency and percentage for categorical variables.

RESULTS

Sociodemographic Details of Participants

Thirty pharmacists were invited to participate in the study, of whom 25 responded. Five participants withdrew: two declined to discuss the subject, and three refused to have their responses recorded. Among the recruited participants, 16 were female (64.0%). In terms of work experience, eight participants (32.0%) had two to five years of experience. The majority (n=17, 68%) were staff pharmacists (employees), while the remaining eight (32%) were pharmacy owners. Participant locations were categorized into two regions: the northern region including Irbid, Ajloun, Jerash, Zarqa, and Mafraq governorates, and the southern region including Balqa, Madaba, Amman, Tafilah, Maan, Aqaba, and Karak. Additional demographic details are provided in Tables 1 and 2.

Almost all participants confirmed that they had engaged in discounts in community pharmacies and did not adhere to medication pricing policies. Three major themes emerged and were classified as: patient-related, pharmacist-related, and rules-and-regulations-related. Each theme was classified into sub-themes, which are presented in Table 3 and Figure 1.

Emerging Themes

Theme 1: Patient-related factors

Patient-related factors can be assessed by looking at the two subthemes which have emerged through the analysis, namely psychosocial factors and economic factors.

Table 1: Sociodemographic characteristic of the participants

Variable	Number	Frequency (%)
Gender		
Male	9	36%
Female	16	64%
Work experience (years)		
Up to 1 year	4	16%
2–5 years	8	32%
6–10 years	3	12%
11–15 years	10	40%
Living area		
North	14	56%
South	11	44%
Position		
Owner of a pharmacy	8	32%
Ordinary employee	17	68%

Table 2: Demographic characteristic of the participants (n=25)

Participant ID	Gender	Work experience (years)	Position
FS 1	Male	5	Employee
FS 2	Male	4	Employee
FS 3	Female	1	Employee
FS 4	Male	3	Employee
FS 5	Female	12	Owner
FS 6	Female	13	Owner
FS 7	Male	1	Employee
FS 8	Female	2	Employee
FS 9	Male	15	Owner
FS 10	Female	4	Employee
FS 11	Female	7	Employee
FS 12	Male	1	Employee
FS 13	Female	8	Employee
FS 14	Female	5	Employee
FS 15	Male	1	Employee
FS 16	Female	3	Employee
FS 17	Female	9	Employee
FS 18	Male	4	Employee
FS 19	Female	11	Owner
FS 20	Female	14	Employee
FS 21	Female	13	Owner
FS 22	Female	15	Owner
FS 23	Female	11	Employee
FS 24	Female	13	Owner
FS 25	Male	14	Owner

Table 3: List of themes and sub-themes that affect Adherence of medicines' prices

Main themes	Sub-themes	Selected quotes
Patient-related Factors	•Psychosocial Factors	"If there is no discount on medicines in the pharmacy, the patient would say I will find a discount in other pharmacies, which is my right", FS 2, FS11. "The pharmacy profit is high; there is no discount on medicines?" Patient asked the pharmacist FS 18, FS 30. "I am your colleague in the medical profession. There is no discount for fellow medical professionals on medicines?". Patient said to pharmacist, FS 11, FS 12.
	•Economic Factors	"I suffer from chronic diseases and I take several medicines. Is there a discount on treatment? Because I do not have health insurance to become a customer of the pharmacy.". patient said, FS 20, FS 22. "I am a patient suffering from reproductive problems and I want IVF treatments, and the cost is high. Are there any discounts?" patient said, FS24.
Pharmacist-related Factors	•Attract Customers, increase profit	"I make discounts in order to win customers, and if I make a one-time discount, the patient will ask for the discount every time, so he would tell me that you discounted my drug the previous time, so you have to make the discount this time as well", FS 21.
	•General Selling Tactics and Market-Share	"The prevailing idea among pharmacies is to make discounts, so I have to make a discount", FS 24. "I make discounts on medicines to attract customers", FS 19.
	•Avoiding Accumulated Medicines	"In the event of a stack of medications, and the company does not guarantee the exchange of this medication close to the expiration date, I have to make a deduction to get rid of this medication", FS 23.
Rules and regulations related factors	•Effective Mandating	"My belief is that the factor of tightening control over the policy of pricing and standardizing medicines is important so that if the patient goes to more than one pharmacy with the aim of pricing a medicine and finds that all pharmacies agree on one price, it will assure the patient that there is no discount from community pharmacies",FS1.
	•Rule of Law	"The application of the law to all violation of the Jordanian drug pricing policy", FS 17.

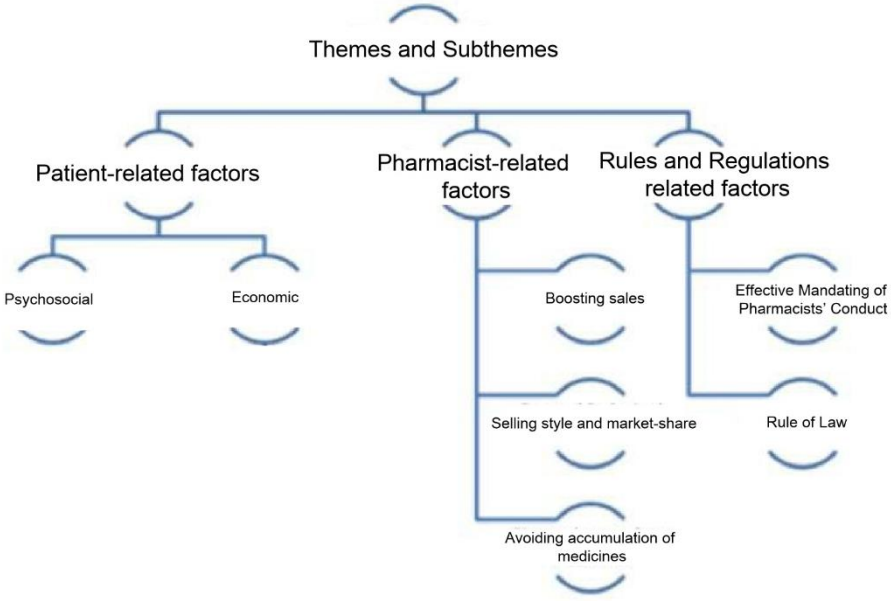

Figure 1: Schematic representation of themes and subthemes that affect adherence to medicines' pricing policies

1.Psychosocial factors:

Participants emphasized that psychological factors significantly influence patients' demand for discounts in community pharmacies. The Jordanian public perceives drug prices as high and believes pharmacists make substantial profits from direct-to-consumer sales. This perception encourages patients to seek discounts. Additionally, participants noted that consumers in Jordan are accustomed to receiving price reductions due to the competitive nature of the pharmaceutical market, reinforcing their expectation of discounts. One participant (FS 21) remarked, "*I struggle to adhere to drug pricing policies because once a discount is given, patients expect discounts in future transactions*."

***2*. Economic Factors:**

Participants highlighted economic factors as crucial in the decision to offer discounts in community pharmacies. Patients' limited income, common in a middle to low-income country, often prompts requests for medication discounts. Another participant (FS 21) noted, "*Both pharmacists and patients face challenging economic conditions*." Furthermore, patients managing multiple chronic conditions with several prescriptions per month, especially without insurance coverage, are more likely to request discounts. Pharmacists respond to these economic pressures to retain their clientele.

Theme 2: Pharmacist related factors

Attracting more patients and boosting pharmacy sales, the general readiness for community pharmacists to give discounts to enhance their pharmacy's market-share, and the desire to liquidate inventories are several subthemes emerging for this theme and are possible factors for a pharmacist to willingly give discounts in community pharmacies.

1. **Attracting more patients and boosting pharmacy sales:**

Pharmacists aim to attract patients and appear more reliable and sociable by giving discounts in pharmacies to increase the net profit that returns to the owner of the pharmacy. One participant (FS 11) said: "*for a pharmacist to have distinct reputation and marketability, one should engage in giving discounts*". This is especially true when a

staff pharmacist is hoping for a raise, or an incentive/commission-based reward added to his/her salary.

2. General Selling Tactics and Market-Share:

Most pharmacies give out discounts to patients on medicines with the intention of increasing the size of their pharmacies' market-share, which in turn puts pressure on other pharmacies to give discounts on medicines thus making it a norm amongst community pharmacists. "*The main advantage of buying medications from a community pharmacy is receiving discounts*" FS4 shared.

3. Avoiding accumulated medicines:

Sometimes pharmacists are inclined to give discounts to avoid having frozen capital and accumulating inventory. "*In the event of a stack of medications, and the company does not guarantee the exchange of this medication close to the expiration date, I have to make a deduction to get rid of this medication"* FS 23 shared.

Theme 3: Rules and regulations related factors

Factors related to rules and regulations of drug discounting are: 1) effective mandating of pharmacists' conduct and 2) justice and law enforcement. Those two factors emerging as subthemes are crucial to unifying drug prices in community pharmacies.

1. Effective Mandating of Pharmacists' Conduct:

Authorities bear the responsibility of tackling the burden of fluctuating prices in accordance with set rules that are committed to transparency and effectively respond to various forces that participate in these operations i.e. consumerism and drug safety. They also bear professional and ethical responsibility for applying these laws. "*My belief is that the factor of tightening control over the policy of pricing and standardizing medicines is important."* FS 1 shares.

2. Rule of Law

The situation in Jordan for discounting medicine prices in community pharmacies has brought on detrimental consequences. Community pharmacists compete with each other trying to undercut prices. Those tactics contribute to the frequency and severity of discount pricing and price competition. Most respondents were found wanting the problem to be addressed.

"The application of the law should be to all violations of the Jordanian drug pricing policy", FS 17 said. Our respondents advocate for a strict rule of law in enforcing minimum pricing strategies, emphasizing the need for stringent measures against violations to ensure fair and ethical business practices within the pharmaceutical industry.

DISCUSSION

Medicines play a crucial role in healthcare. Proper usage and compliance with pharmacotherapy are cost-effective elements of modern healthcare (29), contributing to better quality of life and improved health outcomes. The Jordan Food and Drug Administration (JFDA) is an independent body that governs national retail prices for drugs in the kingdom. It is responsible for continuous assessment of medication prices and sets a fixed income margin for drug manufacturers and importers (30). Pharmacists in Jordan are prohibited from offering discounts on the retail price of medicines. It is noteworthy that patients in Jordan are not legally subjected to pharmacy prescription charges, which are an important source of income for pharmacists globally, from which they are exempt (31).

Although adherence to drug pricing policies in Jordanian community pharmacies is mandatory, the high cost of drugs remains one of the biggest obstacles in Jordan's healthcare system. This study identified several barriers that prevent pharmacists from strictly adhering to the law and from rejecting patient requests for discounts.

Factors Affect Discounting of Medicines' Prices in Community Pharmacies

Patient-related Factors

Patient-related factors contain the sub-themes: psychosocial factors and economic factors.

Given the rising burden of providing affordable drugs, there is significant pressure on people seeking quality healthcare and medication. This is why many patients try to negotiate treatment and medication prices to alleviate

the economic burden they often face (4).

1. Psychosocial Factors

The subthemes identified as psychosocial include personal expectations of service and perceptions of professional status. A study conducted in the United Kingdom affirms that factors influencing pharmacy selection are regular access to quality pharmaceutical advice and professional services, rather than cost and medication promotions (32). In contrast, our study participants (FS 2, 11, 12) expressed a belief in a "right" to discounts based on their medical profession, or a sense of unfairness when pharmacies offer discounts selectively, highlighting cost-related and psychosocial expectations.

Similarly, research on community pharmacy practice in Malaysia indicates that over 85% of participants reported that the prevalence of discounts influences patient loyalty, a psychosocial behavior (21). Literature also suggests that acknowledged discounts positively affect patient confidence, happiness, and satisfaction (33).

2. Economic Factors

In developing countries like Jordan, patients' economic status is a significant factor influencing their choice of pharmacy. Price sensitivity is crucial, especially in the absence of health insurance and with the prevalence of polypharmacy. Patients prefer pharmacies offering the lowest prices, posing a dilemma for pharmacists adhering to drug pricing policies versus risking losing sales. Our study found patients (FS 18, 30) requesting discounts due to perceived high pharmacy profits. Consistent with our findings, Mathews et al. reported that 87% of respondents felt pharmacists may be seen as profiteering (21).

A study in Poland identified fair pricing (78%) and discounts on medications (66%) as primary factors influencing pharmacy choice (32). Similarly, a Greek study categorized 16% of participants as "convenience and price-sensitive customers," with price being a key factor in pharmacy purchases (34). FS24 requested discounts due to the high costs of IVF treatment, aligning with findings in Thailand where customers choose pharmacies based on medication prices (35). Additionally, in Jordan, Ghattas et al. identified sales promotions, including discounts, as statistically significant factors influencing pharmacy selection (36).

Pharmacist-Related Factors

This theme encompasses several sub-themes, including attracting more patients and boosting pharmacy sales, employing general selling tactics to increase market share, and the desire to liquidate stock units. These factors drive pharmacists to agree to giving discounted prices in community pharmacies.

Our study reveals that pharmacists may deviate from drug pricing policies to avoid profit loss in community pharmacy settings. Participants reported a prevalent practice in Jordan where pharmacies offer discounts to attract customers and increase sales, as seen with FS24. FS21 confirmed that discounts are used to attract customers, but once initiated, patients consistently expect discounts regardless of fixed pricing, negatively impacting profitability and credibility. This reflects the concept of Competitive Pricing Strategy in community pharmacy to enhance sales (37).

Encouraging patients to take advantage of set-price, product-based markets relative to competition, discounts are an effective tactic. These findings are consistent with a study in Malaysia where new community pharmacists adjust prices to remain competitive (21). Medicine pricing was identified globally as a key factor in repeat sales, aligning with our study's findings. In contrast, a Dutch study suggests patients are less likely to switch pharmacies based on price alone (38).

Pharmacists continually strive to provide trustworthy, reliable, and professional service to foster customer loyalty and expand market share. While this may diverge from traditional pharmacy roles, it aligns with findings from the Malaysian study (21). However, as retail businesses, offering discounts and promotions on medicines enhances marketability and provides a competitive edge.

Medicines are unique products with expiration dates

crucial to patient desirability and effectiveness. From a revenue efficiency perspective, concern arises that unsold medicine units nearing expiration lose capital value, potentially leading to significant profit loss, as noted by FS23 (33) (34).

Regulations and Law Related Factors:

Medicine pricing policies in Jordan are based on international standards that reference retail prices in various countries, distinguishing between originator and generic brands. The role of the Jordan Food and Drug Administration (JFDA) in these policies is crucial, aiming to mitigate the negative impacts of discounting while meeting the healthcare needs of the Jordanian public. The pricing guidelines must remain dynamic and adaptable to evolving economic and social circumstances, akin to minimum selling price (MSP) practices or price-capping.

Medications are essential public goods that should not be subject solely to market forces such as supply and demand. Therefore, the procurement of medicine and provision of health services should be governed by unified laws or regulatory frameworks rather than individual discretion.

In Jordan, pharmacists often receive compensation around or below the minimum monthly wage set by the Jordan Pharmacists Association (JPA), which is JD350. This stands in contrast to practices in other countries where pharmacists are increasingly compensated separately for dispensing services, proportional to the quantity of items in a prescription (e.g., United States, Australia, Canada). This underscores the need for Jordan to review and establish new laws and regulations to enhance pharmacists' income, mitigate the consequences of discounting, and improve livelihoods.

Significantly, the importance of strict pricing provisions and standardization of medicines was highlighted by nearly all participants. FS1 emphasized the need for such measures to discourage patient requests for discounts. This aligns with findings by Mathews et al., who advocate for healthcare authorities to address pharmaceutical costs and implement MSPs to ensure fairness among community pharmacists. Survey participants suggested that introducing price control protocols could effectively enhance pharmacy practices (21).

Limitations

This study faces a significant limitation due to its small sample size, which restricts the generalizability of findings to all community pharmacists in Jordan. However, the qualitative design allowed for determining the final sample size based on thematic saturation. Despite careful preparation of the interview guide and personalized data collection, complete elimination of recall bias is impossible.

To reduce response burden, minimal demographic information was collected. Including additional demographic data (such as age and educational level) or practice site information (such as weekly prescription volume and pharmacy revenue) could have provided a more comprehensive understanding of community pharmacists' perceptions. Furthermore, the convenience sampling method used introduces potential response bias into the analysis.

CONCLUSIONS

Pharmacy practice and well-established, data-driven drug pricing guidelines can play a crucial role in achieving universal affordability, improved accessibility, and availability of essential medicines.

Our study provides valuable insights for public authorities tasked with managing the challenge of fluctuating drug prices through transparent guidelines and responsive legislation that considers the perspectives of stakeholders such as patients, suppliers, and the pharmaceutical industry. These findings will assist policymakers in developing and enforcing laws governing drug pricing and final retail prices in community pharmacies across Jordan.

The study identifies three major themes: Patient-

related factors, Pharmacist-Related Factors, and Discounts Regulation and Law Related Factors. Subthemes include psychosocial and economic factors, strategies to attract customers and increase pharmacist profits, general selling tactics, market share considerations, and inventory management practices—all of which potentially influence adherence to drug pricing policies in Jordan.

In a price-sensitive environment, it is crucial to further investigate variations in drug pricing practices within pharmacy settings to ensure fairness and mitigate cost-related non-adherence, which could lead to adverse health outcomes. Several avenues for future research are suggested. Qualitative studies could explore the systematic practice of drug price discounting across different products in community pharmacies. Additionally, quantitative research could examine the relationship between discounting frequency and factors such as the density of competing pharmacies or regional demographics.

Appendix 1: Interview Guide

Participant information
Gender
Number of years of work
Pharmacy location
Pharmacist job (Staff/Owner)

Questions related to compliance with the drug pricing policy:

1. In your opinion, what are the advantages of offering the public a discount on drug prices?
2. In your opinion, what are the disadvantages of offering the public a discount on drug prices?
3. What are the motives that prompt pharmacists to offer discounts on drug prices?
4. What are the obstacles and difficulties facing the pharmacist regarding offering drug price discounts?
5. Why do you think the public requests discounts on medicines?
6. In your opinion, how effective is the law that prevents discounts on medicines? Should it be modified?
7. In your opinion, what benefit does a pharmacist gain from the discounts offered to the public on medicine?

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وجهات نظر صيادلة المجتمع حول تقديم أسعار مخفضة لأدوية الوصفات في الأردن

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ملخص

في الأردن، يتم تسعير الأدوية من قبل مؤسسة الغذاء والدواء الأردنية (JFDA) التي تلزم الصيادلة ببيع المنتجات الصيدلانية دون أي تخفيضات أو حسومات. ومع ذلك، فإن خصم أسعار الدواء في صيدليات المجتمع أمر شائع. تهدف الدراسة الحالية إلى فهم دوافع و توجهات الصيادلة المؤدية لتخفيض أسعار الأدوية في صيدليات المجتمع في الأردن. تم إجراء المقابلات النوعية مع عينة مكونة من 20 صيدلاني من صيادلة المجتمع. تمت مقابلة 25 مشاركًا، مع إخفاء هويتهم، وتم تسجيل المقابلات صوتيًا. ولتحقيق أقصى قدر من الراحة، أتيحت للمستجيبين فرصة حضور المقابلة في مكان من اختيارهم أو عبر أدوات التواصل السمعية والبصرية. تم إجراء المقابلات باستخدام دليل مصمم مسبقًا. يناقش دليل المقابلة تقديم تخفيضات على الأدوية الموصوفة في صيدليات المجتمع وتجارب الصيادلة ومواقفهم على هذا الصعيد. تم نسخ تسجيلات المقابلة وتحليلها موضوعيا.

النتائج: ظهرت ثلاثة محاور رئيسية، بما في ذلك العوامل المتعلقة بالمريض، والعوامل المتعلقة بالصيدلاني، والعوامل المرتبطة بالقوانين واللوائح، ولكل منها بعض المواضيع الفرعية. بينت هذه الدراسة عدة عوامل أعاقت التزام الصيادلة بسياسات تسعير الأدوية في صيدليات المجتمع في الأردن منها العوامل النفسية الاجتماعية، العوامل الاقتصادية، جذب العملاء وزيادة ربح الصيدلي، أساليب البيع، زيادة الحصص السوقية، تجنب تراكم الأدوية وانتهاء صلاحيتها وإنفاذ القانون. نستنتج من النتائج التي تم جمعها أن الالتزام بسياسات تسعير الأدوية في صيدليات المجتمع في الأردن ليس مثاليا ولكنه يعتمد على عوامل مختلفة. وبالتالي، قد يُظهر الصيادلة التزامًا ضعيفًا في ظل ظروف معينة بينما يكون لديهم التزام افضل في ظل ظروف أخرى. تقدم هذه الدراسة رؤى لإعادة النظر في السياسات والقوانين المتعلقة بممارسة الصيدلة والالتزام بتعليمات التسعير.

الكلمات الدالة: التسعير، الخصم على الأدوية، صيدليات المجتمع، الأردن، المؤسسة العامة للغذاء والدواء.

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رقم الإيداع لدى دائرة المكتبة الوطنية
(2008/23.3/د)

عمادة البحث العلمي

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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