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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

Letter from the Editor-in-Chief

We all hope that this year would be the end of the pandemic, so life will start again. We started -although slowly- getting back to normal life. Teaching and meetings are again face to face, and researchers are again working together. Jordan Journal of pharmaceutical Sciences (JJPS) is not an exception; our editorial team enjoyed face to face discussions, selecting reviewers and taking decisions related to research works after those hard times working completely online before. JJPS continues to publish the 4 issues of (JJPS) on regular times: one issue every quarter with 10 accepted articles per issue. Despite the enthusiasm, ambition and optimistic teamwork of the editorial team, challenges are still being faced; particularly waiting time from submission till sending a decision to the researcher. One of the main obstacles that causes the delay is the electronic system of submission, tracing and evaluation, as most researchers, reviewers and editorial members are suffering from the current user-unfriendly system. Meetings of the Jordanian journals' Editors-in-chief with the administrative and technical people in the Deanship of Scientific Research led to a promise for introducing a completely new electronic system that will make life much easier for researchers, reviewers, editorial board members and even the editorial working team. The latter just finished its trial version with good feedback so far. JJPS people are looking forward to having this new faster system implemented soon hoping that the second issue for 2022 will be fully and easily practiced by all .

JJPS teams started already to classify reviewers according to their time of response to the reviewing process, working with (A) class reviewers would decrease times for researchers who submitted their work to the JJPS waiting for the feedback. In general, we have distinguished colleagues from more than 30 universities in Jordan representing all scientific pharmaceutical domains and with a diversified experience: recent comers from well-known high ranking world universities as well as wise experienced current available scientists .

The University of Jordan recently agreed its new financial budget for 2022; the good news is that the scientific research budget allocated this year is double than the previous year. Which hopefully would reflect on the quality of the research performed and subsequently published for the academicians in the region.

Prof Ibrahim Alabbadi
Editor-in-Chief

Editorial Commentary

Dear Researchers,

Pharmaceutical care has been the core of pharmacy research for the last three decades. The shift in pharmacy practice from a product-oriented profession into a patient-oriented profession has been reflected on pharmacy research and patient centered , pharmacy research has been on the rise since then.

Though the introduction of the pharmaceutical care concept was initiated in the early 90s by (Hepler and Strand), it took more than two decades to be researched in Jordan. Reports of early Pharmceutical care research in Jordan go back to 2003. This was followed with a gradual restructuring in pharmacy curricula in various faculties of pharmacy in Jordan and the introduction of PharmD programs and MSc in clinical pharmacy.

Recently, the accreditation requirements for pharmacy programs have been changed with clear focus on pharmaceutical care. Furthermore, pharmaceutical care has been researched extensively in various settings. Studies focused on pharmaceutical care in hospitals and community pharmacies, reporting that the change in pharmacy education has not been reflected on practice, and that pharmacy practice in Jordan needs robust reform to match the change in education.

Researchers are encouraged to study the predictors of resisting change in pharmacy practice in Jordan. Further research tackling this matter could help implement patient-oriented practice and harbor optimal treatment outcomes leading to cost effective treatment choices.

JJPS encourages fellow researchers and colleagues to submit their research in pharmaceutical care and similar fields for potential publication in its coming issues.

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CONTENTS

Jordan Journal of Pharmaceutical Sciences, Volume 15, No. 2, 2022

Extraction of anthocyanins from *Clitoria ternatea L.* petals in Vietnam and determination of its antioxidant and antimicrobial activities

Vy T.N. Ha[1], Ngoc T.H. Le [1*]

[1]School of Biotechnology, International University-Vietnam National University, Vietnam.

ABSTRACT

In this study, the effects of various factors on anthocyanins extracted from *ClitoriaTernatea L.* petals were determined. In addition, phytochemical properties, antioxidant ability, antimicrobial capacity, and application of anthocyanins extractions were also investigated. As a result, the highest proportion of anthocyanin, 1.21 mg/g fresh weight (FW), was obtained when extracting *ClitoriaTernatea L.* petals with 100% methanol, with the solvent/sampleratio of 10ml/g at 37°C for an hour. The highest total phenolic content (TPC) was $24.7x10^3 \pm 8.55x10^2$ µg gallic acid equivalent (GAE)/g FW, while the figures for total flavonoid content was 18.8±0.149 mg quercetin equivalent (QE)/g FW. The values for 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity and the highest total antioxidant activity were EC_{50}=904.1µg/ml and $2.86x10^3 \pm 1.01x10^1$ µg ascorbic acid equivalent (AAE)/g FW, respectively. The inhibitions to *Staphylococcus aureus*, *Streptococcus mutans,* and *Pseudomonas aeruginosa* were shown with the minimum inhibitory concentration (MIC) of 90 mg/ml and 180 mg/ml. It also showed that the *ClitoriaTernatea L.* petal extract also had the potential to be developed into a pH indicator, however, further study should be carried out to obtain a highly qualified one.

Keywords: *Clitoria Ternatea L.* petal, anthocyanins, optimization, pH indicator, phytochemical properties.

INTRODUCTION

Clitoria Ternatea Linn – first called by Breyne, common named butterfly pea, is a plant belonging to a vine family, *Fabaceae*. The flower of *Clitoria Ternatea L.*, which can be blue or white color, is reported to contain numerous substances. Soluble minerals and soluble carbohydrates with high concentration, phenolics and flavonoids were found this kind of flower. Anthocyanins, the substances that contributed to the blue color of the petals, have been found. They are the acylated form, which has potentially high stability,

In addition to being used as food colorant and dyes, the flower has been used for the ornamental purpose all over the world. It is also proven for pharmacological activities and used as an Ayurvedic medicine for thousands of years. Its extract showed significant results in anticancer to different cell lines using the trypan blue dye exclusion method. The blue line flower also showed remarkable antioxidant activity to 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) [1-5].

Anthocyanins, which are one of the flavonoids' sub-groups, are water-soluble natural colorants. They contribute to the majority of pigments discovered in plants from orange, red to blue. They can be predominantly found in fruits of berries, apples, grapes, and others, flower petals, and partly found in other parts of the plants namely root or leaves [6]. The anthocyanins obtained from different sources are also of great variance. In addition to increasing

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animal attractions, anthocyanins also benefit human's health. They have been claimed for the potential of being food colorants and food additives due to their safety and high stability, while other articles have pointed out that they could show inhibition to cancer and chronic symptoms. Anthocyanins are easily affected by pH, light, temperature, metals, copigmentation and other substances [3,6]. Based on the effect of pH on anthocyanins color, pH indicators developed from its extract was considered [7].

Clitoria Ternatea L. flower is usually used as food colorant in addition to the use as tea ingredient. There is no official document for the Vietnamese traditional used of this flower published internationally.

The essence of this study was to investigate in the total anthocyanin content at the optimal condition of *Clitoria Ternatea L.* flower growing in Vietnam. Several characteristics of the extract at this condition included total phenolic content, total flavonoid content, antioxidant and antimicrobial activities were also determined. The pH sensitivity of this petal was also evaluated in order to develop a pH indicator.

Materials and methods

Materials

After pretreatment, only the *Clitoria Ternatea L.* petals collected in Phuong Lam, Dong Nai province, Vietnam were kept and stored at -20ºC until performing the experiment.

Screening for anthocyanins sources

Fresh and dried samples were screened for their anthocyanin content [8,9]. The samples were macerated in 100% methanol containing 0.1% (v/v) concentrated hydrochloric acid with a ratio of solvent/sample of 5ml/g at 37ºC for 1 hour. The extracts were then centrifuged and diluted with the mixture of ethanol: 1.5N hydrochloric acid (85:15 v/v). The absorbance at 535 nm was then measured by the followed formula and expressed as mg total anthocyanin content (TAC)/g fresh weight (FW):

Total anthocyanin content (TAC) (mg/g FW) =

$$\frac{A_{535nm}\ x\ dilution\ factor}{98.2\ x\ weight\ of\ fresh\ sample}$$

In which: A_{535nm} was the absorbance of the solution, 98.2 was the extinction coefficient value of cranberry anthocyanin in the mixture of ethanol and hydrochloric acid (mg/ml).

Components of the extracts were then analyzed using thin-layer chromatography (TLC) technique on cellulose plate and Forestal, glacial acetic acid : hydrochlori c acid : water (30:3:10), as the mobile phase [19].

Optimization of anthocyanins extraction

In this part, the effects of several parameters, including time of extraction, temperature, solvent/sample ratio, types of solvent and solvent concentration, on TAC would be studied**.** Sample containing a higher total anthocyanin proportion in the previous part was used to optimize the anthocyanins extraction. The extraction would follow the modified methods [8,9]. The sample was initially extracted with 100% methanol containing 0.1% (v/v) concentrated hydrochloric acid with a ratio of solvent/sample of 5ml/g at 37ºC for various durations of extraction. The optimal condition of one factor would be subsequently used to study the following factors. Its TAC was determined as mentioned in the previous part to determine the optimal conditions for each factor.

Characterization of the extract of Clitoria Ternatea L. petals

TPC, TFC, DPPH scavenging capacity and total antioxidant activity test procedures were adopted from Yadav and colleagues' work in 2017 with some modifications [10].

Determination of total phenolic content (TPC)

A 250µL aliquot of Folin-Ciocalteau's reagent and 250µL of 7.5% sodium carbonate solution were added to 100µL Gallic acid solutions (6.25-100 µg/mL) and incubated for 30 minutes at room temperature (RT) before measuring the absorbance at 765 nm. The process was applied to the extracts. Their TPC was expressed as µg GAE/ g FW.

Determination of total flavonoid content (TFC)

A mixture of 100µL of the Quercetin solution (0.75-10. mg/ml), 300µL methanol, 20µL of 10% Aluminum Chloride, 20µL ml of potassium acetate and 560µL of distilled water

was incubated at RT for 30 minutes, and then measured at 420nm. The procedure was applied to the extracts to evaluate the TFC of the extract expressed as mg QE/g FW.

Determination of antioxidant capacity

DPPH scavenging capacity: An amount of 0.75ml of 0.0067% DPPH solution was allowed to react with 0.25ml of extract or L-ascorbic acid at various concentrations for 20 minutes. The absorbance was measured at 517nm. The results were displayed as EC_{50}.

Total antioxidant activity: An amount of 0.1 ml of ascorbic acid (6.25-100 µg/mL) was allowed to react with 1ml of reagent solution at 95ºC for 90 minutes followed by absorbance measurement at 695nm. The same process was applied to the extracts. Its total antioxidant capacity was determined and then represented as µg AAE/g FW.

Determination of antimicrobial activity

Antimicrobial activity of the extracts was evaluated by employed procedure with modifications[11,12]. Discs containing the extracts with the concentration of 180 mg/ml were utilized to determine antimicrobial activity on plates spreading bacteria, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, while appropriate antibiotics were used as positive control. MIC was determined using discs containing extracts with various concentrations. The diameters of the clear zones were used in order to determine the antimicrobial power as well as the MIC of the extracts after overnight incubation at 37ºC.

Determination of pH sensitivity

pH indicators was developed as described by Syahirah L et al. (2018) [13]**.** For liquid pH indicator, color changes in pH 1 to 13 solutions after adding water extract of *Clitoria Ternatea L.* were recorded. While colors of pH paper containing the extract with pH 1 to 13 solutions was compared with those developed on the universal pH test paper strips.

Statistical analysis

All the experiments were triplicated. The results were then analyzed with t-test or ANOVA one-way and Turkey's Test using SPSS.

Results and Discussion

Screening for anthocyanins sources

The chart displayed the fresh sample had a statistically higher potential of TAC (1.056±0.0361 mg/g sample) than that of dried sample ($0.319\pm9.07\times10^{-3}$ mg/g sample) with an equivalent proportion, **Figure 1** ($p<0.05$). When analyzed with TLC, the fresh sample was confirmed to contain more compounds with higher concentration compared with the dried one. Their R_f was approximately similar, which was 0.813, **figure 2**.

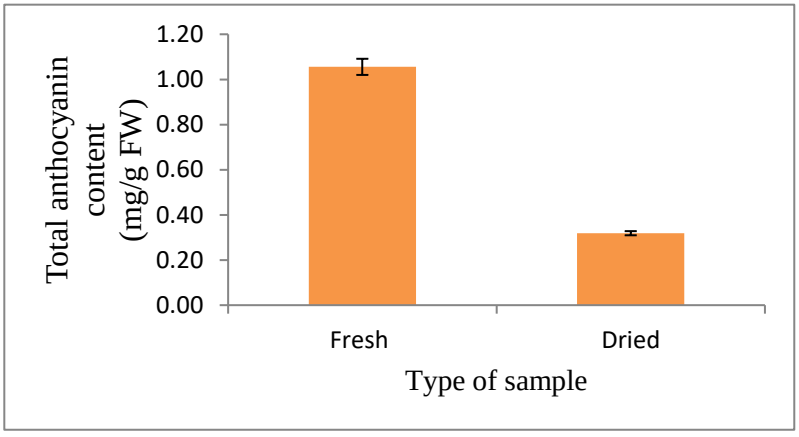


Figure 1. Total anthocyanin content of the fresh and dried samples

Figure 2. TLC result of (F) fresh sample and (D) dried sample

The difference could be caused by the pretreatment of samples. While the fresh petals were collected and stored at -20°C, the petals were dried at 40°C and ground. This treatment process might have significantly destroyed the TAC in the sample, which sensitive to temperature [3]. The brighter and broader band for fresh sample and the narrow and pale band for the dried sample in TLC, even though both bands had the same R_f value, indicating that the fresh sample had more anthocyanins with higher concentrations. Thus, fresh petals of *Clitoria Ternatea L.* were selected.

Optimization of anthocyanins extraction

As shown in **figure 3**, the sample extracted for 1 hour showed the highest yield of anthocyanins ($p<0.05$), followed by that of extraction for 1.5 hours. The optimal time extraction was similar to the one mentioned in a study in 2008 [14], however, was different from other research. The optimal duration for anthocyanins extraction was 1.5 hour [8,15], whereas it was reported to be up to 2.5 hours [16]. The extraction time of one hour was considered as the optimized time in order to evaluate other factor effects.

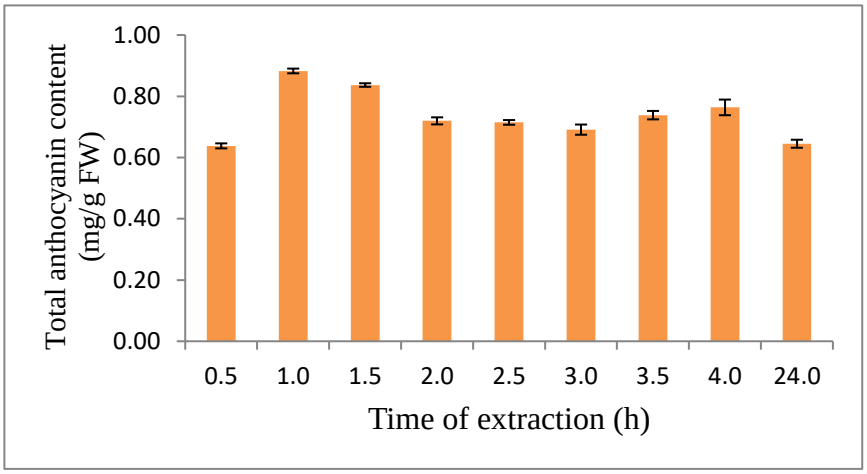


Figure 3. Total anthocyanin content of the fresh sample under the effect of time extraction

The optimal time extraction was applied with other initial conditions to investigate the effect of temperature on TAC. An increase in temperature from 4ºC to 37ºC resulted in an increase in TAC, the TAC extracted at 37ºC was the highest one (0.883 ± 7.64 x 10^{-3} mg/g FW) (P<0.05), **figure 4**. However, this acceleration was reversed when the temperature continued to reach higher, there was no significant difference among total anthocyanin content extract at other temperatures (p<0.05). This could be explained by the hydrolyzation of 3-glucoside structure and pyllirium ring [17]. The effective temperature for anthocyanins extraction about 35ºC was reported [15], meanwhile the study in 2008 concluded that extraction at 80ºC would bring the highest yield [14].

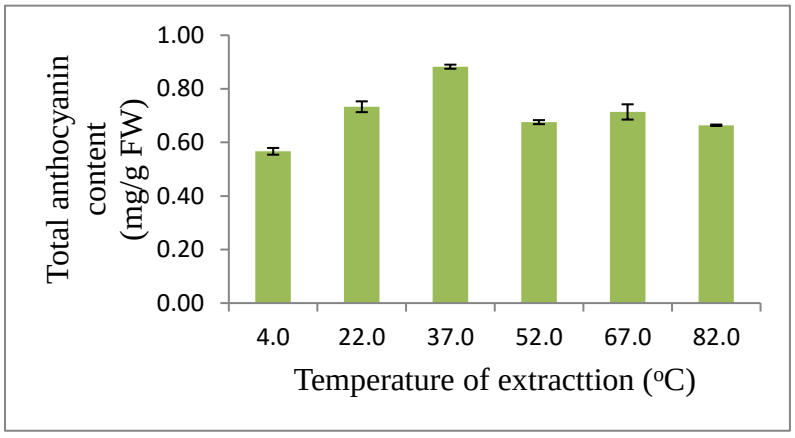


Figure 4. Total anthocyanin content of the fresh sample at different temperature

The effect of solvent/sample ratio, which was reported to affect the efficiency of anthocyanins extraction according to Cacace & Mazza (2003) [15], was identified under time extraction of 1 hour at 37ºC. The indifferences between extracts with different ratio of solvent/sample, except for the one extracted with the ratio of 10 ml/g, the highest one (1.21±4.61x10^{-2} mg/g FW) (p<0.05), **figure 5**, led to a conclusion that a reduction in TAC might be because high solvent/sample ratio could have caused further dilution, leading to a drop in the reading. This ratio was similar to the one reported in the study of Blackhall et al. (2018) [8], whereas this ratio was 15ml/g in another study [16].

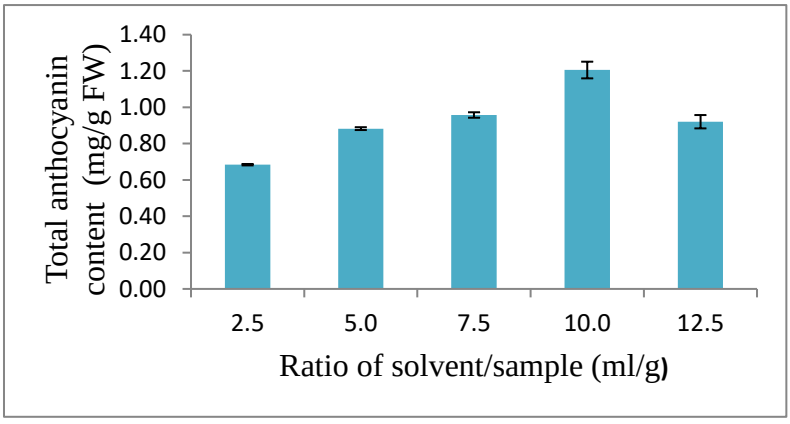


Figure 5 Total anthocyanin content of the fresh sample with variety ratio of solvent/sample

The suitable solvent would give potential result in anthocyanins extraction. Alcohols, especially methanol and ethanol containing low concentration of acid, were advised to be used due to their cell membrane denaturation property and provision a suitable medium for flavylium ions, a form of anthocyanin. Ethanol was preferred due to its safety for human consumption, especially in food industry [18,19]. In **figure 6** methanol containing 0.1% v/v concentrated HCl resulted in high and significant different TAC yield, $1.21\pm4.61x10^{-2}$ mg/g FW in compared to ethanol containing 0.1% v/v concentrated HCl, $0.967\pm1.05x10^{-2}$ mg/g FW ($p<0.05$). The ethanol solution, however, was still being used to identify the optimal conditions for future application in the food industry.

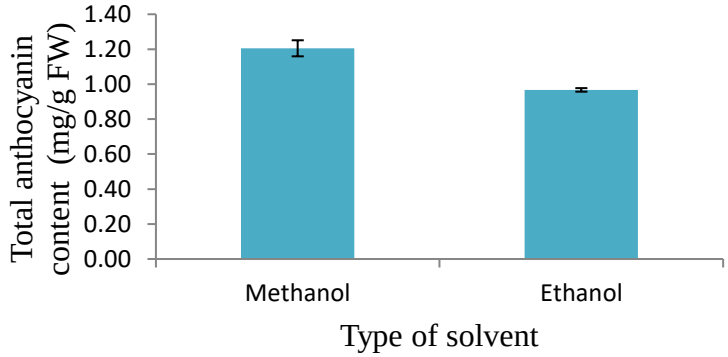


Figure 6. Total anthocyanin content of the fresh sample extracted by methanol and ethanol

The effect of concentration of solvent was carried out, subsequently. Water with various proportions were advised to be added to extract anthocyanins completely [6,18]. **Figure 7** showed that the extract with solution of 100% methanol yielded the highest TAC, followed by the extract with 100% ethanol mixture. There was no significant difference between total anthocyanin content extracting with other concentrations ($p<0.05$). The results were different from previous studies which could be due to different materials used in anthocyanins extraction. The exact explanation for this was still unknown.

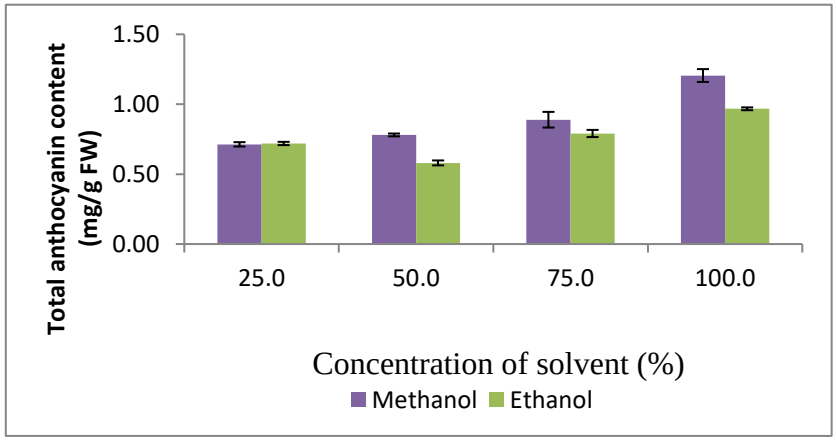
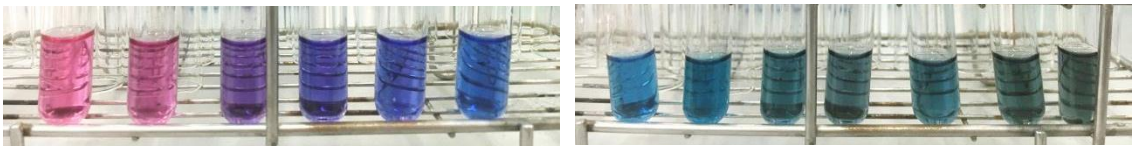


Figure 7. Total anthocyanin content of fresh sample extracted by various concentrations of methanol and ethanol



(a) (b)

Figure 8. Result of liquid pH indicator. (a) Color developed in pH 1 to 6. (b) Color developed in pH 7 to 13

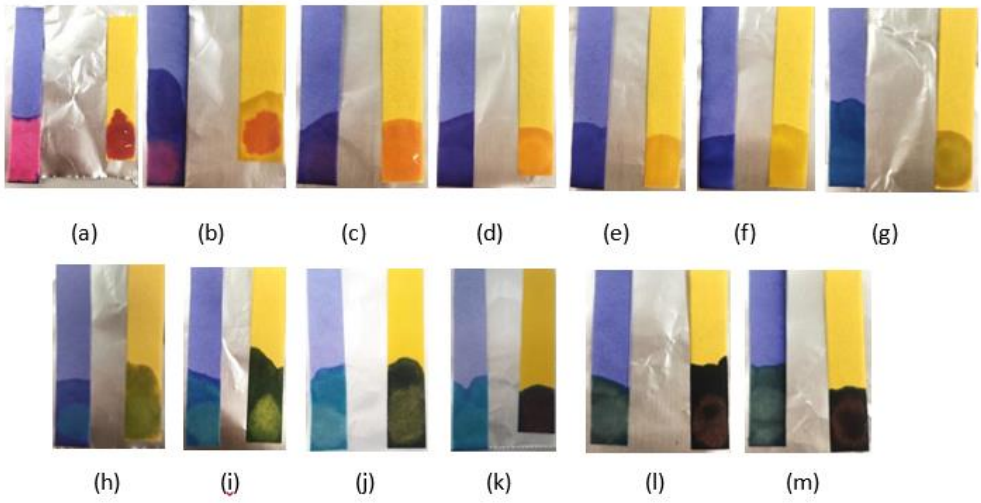


Figure 9 .Color changing indicated by pH paper and universal pH indicator respectively. From (a) to (m) color developed in pH 1 to 13

(a) (b) (c)

Figure 10 Results of antimicrobial activity of methanol extract against *Staphylococcus Aureus*. (a) methanol extract. (b) positive control. (c) negative control. The determination of antimicrobial activities of methanol extract and ethanol extract against other bacterial strains were performed similarly

Consequently, *Clitoria Ternatea L.* petal should be extracted for one hour at 37ºC with the solvent/sample ratio of 10ml/g of 100% methanol with 0.1% concentrated HCl to obtain an optimal amount of anthocyanins. Extract under these conditions using ethanol would yield the most efficient anthocyanins in food industry. Both extracts would then be evaluated for other characteristics in this study.

Characterization of the extract of Clitoria Ternatea L. petal

Determination of total phenolic content (TPC)

Phenolic compounds are compounds that are widely distributed in plants with several properties such as antioxidant, antimicrobial and health benefits [20-21].The means of TPC of two extracts were mentioned in **table 1**. TPC of methanol extract and ethanol extract was calculated from the linear regression equation derived from the standard curve of Gallic acid, $R^2 = 0.9986$. There was significantly higher TPC in methanol extract ($24.7x10^3±8.55x10^2$ µg GAE/g FW)and compare to that of ethanol extract ($18.8x10^3±2.46x10^2$ µg GAE/g FW) ($p<0.05$). This suggested that various types of solvent also affect the phenolic content. In this case, since methanol was more polar than ethanol, solvent polarity could have resulted in different amounts of TPC. These figures were slightly lower than the reported 26.72±2.17 mg GAE/g of dry flower[22] andmuch lower than the reported figure of 76.90 mg GAE/g FEW [20]. However, these data were higher than TPC of other anthocyanins sources, 2087.43±17.37 mg GAE/100 g FW[23] and 294mg GAE/100 g FW[24].

Table 1. Total phenolic content, total flavonoid content and total antioxidant activities of the extracts.

	Methanol extract	Ethanol extract
Total phenolic content (µg GAE/g FW)	$24.7x10^3±8.55x10^2$	$18.8x10^3±2.46x10^2$
Total flavonoid content (mg QE/ g FW)	18.8±0.149	15.5±0.091
Total antioxidant capacity (µg AAE/g FW)	$2.86x10^3±1.01x10^1$	$2.57x10^3±2.50x10^1$
DPPH scavenging capacity (EC_{50}) µg/ml	904.2±0.327	904.0±0.581
Total antioxidant activity (µg AAE/g FW)	$2.86x10^3±1.01x10^1$	$2.57x10^3±2.50x10^1$

Determination of total flavonoid content (TFC)

The proportion of flavonoids, the largest group of plant phenolic compounds [19], was observed in these extracts. The reliable data for TFC of the extracts, which were quantified by the standard curve of Quercetin with $R^2 = 0.9995$ were listed in **table 1**. It revealed that means of TFC of methanol extract was 18.8±0.149 mg QE/g FW, significant different compared to that of ethanol extract, 15.5±0.091 mg QE/g FW. Similar to figure for TPC, the TFC figure of methanol extract was also significantly higher than that of ethanol extract in spite of the same sources which suggested a further study on the effect of solvents on proportion of phenolic and flavonoid in *Clitoria Ternatea L.* The figure for TFC of methanol extract was higher than the reported one, 16.44 ± 0.02 mg QE/g FW [24], while TFC of ethanol extract was slightly lower.

Determination of antioxidant capacity

DPPH scavenging capacity: The DPPH radical was neutralized by the antioxidants, which caused the discoloration measuring at 517 nm. The efficacy of antioxidants was then expressed as EC_{50}, which was the antioxidant concentration that could reduce the concentration of DPPH by 50% [25]. The efficient concentration for scavenging of 50% DPPH (EC_{50}) of both extracts was shown in **table 1.** There was no significant difference between the EC_{50} of methanol extract and that of ethanol extract ($p<0.05$). The results could be affected by technical skills. The EC_{50} was higher than 0.76±0.03 mg/ml [24], and 84.15±1.50 µg/ml[26] which could be blamed on the differences in anthocyanin content and phenolic content. This meant that the Clitoria

Ternatea L. petals collected in Phuong Lam, Dong Nai province had less antioxidant ability than the petals harvested in Malaysia and Thailand, respectively. .

Total antioxidant activity: By measuring the absorbance of phosphomolybdenum complex produced after reducing Mo(VI) to Mo(IV), the ability of antioxidant would be determined [27]. The antioxidant activity of methanol extract ($2.86x10^3±1.01x10^1$ µg AAE/g FW) was presented in **table 1**, while that figure for ethanol extract was $2.57x10^3±2.50x10^1$ µg AAE/g FW ($p<0.05$). Methanol extract had a significantly higher antioxidant ability than ethanol extract. This difference could be from the difference in the proportion of anthocyanins, and phenolic compounds content of these extracts. This was unsurprising and data was consistent with prior findings.

Determination of antimicrobial activity

Methanol extract presented more effective antimicrobial inhibition to *Staphylococcus aureus,* and *Pseudomonas aeruginosa* compared to ethanol extract, except for *Streptococcus mutans* which they showed the same antimicrobial ability ($p<0.05$), **table 2**. The inhibition zones of these extracts were much smaller than those of the appropriate antibiotics. Leong et al. (2017) also reported that *Clitoria Ternatea L.* extract did not show inhibition to Escherichia coli [28]. However, an opposite result was found [2,12]. While MICs against *Staphylococcus aureus*, *Streptococcus mutans* were the same for both extracts, 90 mg/ml, the MIC of ethanol extract against *Pseudomonas aeruginosa*, 180 mg/ml, doubled that of the methanol extract. It could be claimed that both methanol extract and ethanol extract had the same inhibitory strength to *Staphylococcus Aureus,* and *Streptococcus mutans*. Methanol extract presented the greater inhibitory strength to *Pseudomonas aeruginosa* than ethanol extract. The MIC for *Staphylococcus aureus* was lower, the MIC for *Pseudomonas aeruginosa* was higher than the reported one [12]. This could be caused by the use of different strains of the bacterial species and their unknowingly developed resistance.

Table 2.Antimicrobial activities of Clitoria Ternatea L. extracts

	Zone of growth inhibition (mm)		
	Positive control	**Methanol extract**	**Ethanol extract**
Escherichia coli	17.9±0.404	-	-
Salmonella typhi	27.4±0.306	-	-
Staphylococcus aureus	32.2±0.152	15.5±0.351	10.5±0.153
Streptococcus mutans	35.2±0.451	11.4±0.100	11.3±0.208
Pseudomonas aeruginosa	36.0±0.208	11.4±0.322	8.40±0.173

'-' inhibition zone was not recorded

Determination of pH sensitivity

In acidic media, flavyliumcation was predominant, causing the solution to turn red. By increasing the pH, the form of carbinolpseudobase (colorless) of anthocyanins increased while there was a decrease in flavylium form that led to the fade in color at pH 4 to 5. Above pH 5, quinonoidal structure was the dominant responding of the color. This structure would turn to be chalcone structure when pH was over 7. Chalcone, colorless, could contribute to pale yellow color development. However, it was unstable [7,29-32] . The result in color changes for pH over 7 was found to be inconsistent, the reason remained unknown.The petals were extracted with water with pH adjusted to 7. Colors developed in liquid pH indicator showed different shades which could be distinguished from one another, changing from light pink to purple, then blue and turquoise accompanied pH changed from 1 to 11, except for the colors of buffer pH 12 and pH 13, turquoise

hue with the yellowish shade, that could not be distinguished instantly. The yellow shade disappeared immediately after few seconds adding the extract. This observation was quite different from the reported ones by TawadchaiSuppadit (2011) [33], who found the yellowish color at pH 12 and a light yellow color at pH 13, and yellow color at pH 12 by Abdullah and colleagues[34]. On the other hand, the pH paper showed longer lasting and more distinguishable colors for pH 3, 4 and 5 in compared to that of universal pH paper. However, it was better to use universal pH paper to differentiate pH 7 and 8. The pH paper showed clear different colors for pH 12 and 13, which was yellow with dissimilar shades. These colors faded after a few seconds exposed to the atmosphere, which can be caused by the instability of chalcone structure. Further study should be carried out in order to develop a stable pH indicator from natural sources.

Conclusion

In conclusion, the optimal extraction condition of *Clitoria Ternatea L.* petals was determined. The utilization of methanol as solvent yielded more effective result. However, ethanol could be applied to extract anthocyanins in food industry with comparable characteristics. It showed that the extracts also containing other phenolics and flavonoids, had noticeable antioxidant activity and antimicrobial ability. Slight differences between liquid pH indicator and pH paper showed, further study should be performed to develop a more stable and reliable pH indicator from natural sources.

Acknowledgement

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استخراج الأنثوسيانين من بتلات *Clitoria ternatea* L. في فيتنام وتحديد أنشطتها المضادة للأكسدة والميكروبات

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ملخص

في هذه الدراسة تم تحديد تأثير العوامل المختلفة على الانثوسيانين المستخرج من بتلات *ClitoriaTernatea* L. بالإضافة إلى ذلك، تم فحص الخصائص الكيميائية النباتية، والقدرةالمضادة للأكسدة، والقدرة المضادة للميكروبات، وتطبيق مستخلصات الأنثوسيانين .نتيجة لذلك، تم الحصول على أعلى نسبة من الأنثوسيانين، 1.21 مجم / جرام وزن طازج(FW) ، عند استخراج بتلات *ClitoriaTernatea* L. مع100 ٪ ميثانول، مع مذيب / عينة 10 مل / جمعند 37 درجة مئوية لمدة ساعة .أعلى محتوى فينولي إجمالي (TPC) كان 8.55x102± 24.7x103 ميكروغرام مكافئ حمض الغاليك / (GAE) جمFW ، بينما كانت أرقام إجماليمحتوى الفلافونويد 0.149 ± 18.8 مجم مكافئ كيرسيتين / (QE) جم .FW كانت قيم قدرة الكسح2 ،2- diphenyl-1-picrylhydrazyl (DPPH) وأعلى نشاط إجمالي لمضادات الأكسدة هي EC50 = 904.1 ميكروغرام / مل و 1.01x101 ± 2.86x103 ميكروغرام مكافئ حمض الأسكوربيك / (AAE) جمFW ، على التوالي. تم عرض مثبطات Staphylococcus aureusو Streptococcus mutans و Pseudomonas aeruginosa بأقل تركيز مثبط (MIC) يبلغ 90 مجم / مل و 180 مجم / مل .وأظهرت أيضًا أن مستخلص بتلة *ClitoriaTernatea* L. لديه أيضًا إمكانية تطويره إلى مؤشر الأسالهيدروجيني، ومع ذلك، يجب إجراء مزيد من الدراسة للحصول على واحد مؤهل تأهيلا عاليا.

الكلمات الدالة: *ClitoriaTernatea* L. :البتلة، الأنثوسيانين، التحسن، مؤشر الأس الهيدروجيني، الخواص الكيميائية النباتية.

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Antidiabetic activity, polyphenols-based characterization and molecular interaction of extract of un-ripe pods of *Vinca rosea* cv. Pink

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ABSTRACT

The study aimed to investigate extracts of un-ripe pods of Vinca rosea cv. Pink for antidiabetic activity and polyphenol-based characterization of extracts using HPLC. Moreover, the molecular interaction of the identified markers with the antidiabetic targets was explored using molecular-docking software. Different extracts of un-ripe pods were prepared and investigated using antidiabetic models such as glucose uptake by yeast cells, alpha amylase inhibition and haemoglobin-glycosylation inhibition assays. The most active extract was characterized by HPLC using chlorogenic, caffeic and ferrulic acids as an analytical markers. The identified markers were taken as ligands for molecular docking with pancreatic α-amylase, glycogen phosphorelase and hexokinase-I using 1-Click Docking Mcule Software, and finding hydrogen-bonding affinities by UCSF Chimera 1.12. Methanol extracts showed higher antidiabetic activity of 37.77, 71.16 and 53.52% inhibition of α-amylase assay, increase in glucose-uptake by yeast cells, and inhibition of Hb-glycosylation assay, respectively. The extract was found to contain 0.25 mg/g of chlorogenic and 0.11 mg/g caffeic acids. These markers were found to be good ligands of diabetes related targets. The results indicate that methanol extract has antidiabetic activity, which may be assigned to chlorogenic and caffeic acids. These compounds may be used as pharmacological markers to standardize the extracts.

Keywords: Vinca rosea cv. Pink, Antidiabetic activity, Un-ripe pods, polyphenols, Reversed-phase HPLC, Molecular interaction.

INTRODUCTION

Plants, being the source of diverse bioactive compounds, may play a key role in controlling diabetes, which is affecting more than 170 million individuals globally. [1-2] For such purpose, medicinal plants used as folklore antidiabetic medicine need to be explored to find alternative remedies for this alarming disorder. As a folk-remedy, people swallow fresh leaves of *Vinca rosea (*synonym: *Catharanthus roseus* (L.) G. Don) by chewing to control diabetes. The literature also provided evidence that extracts of leaves of the plant had antidiabetic activity. [2-3-4] But, to the best of our literature review, extracts of un-ripe pods of *Vinca rosea* cv. Pink have not been investigated for antidiabetic activity.

Vinca rosea is commonly called Madagascar periwinkle, Rose periwinkle and cape periwinkle belongs to the family *Appocynaceae*. It is an evergreen herbaceous plant, usually 1m in height having long green glossy leaves arranged in opposite pairs on slender stem. Flowers are usually white to rose pink with a darker red centre. The plant is reported to contain a number of phenolic compounds [5-6] some of which (chlorogenic, caffeicand ferulic acids) have been investigated for their antidiabetic action. Wang et al.[7]had investigated the effects of chlorogenic acid on the hepatic glucose 6-phosphatase,blood glucose, skeletal muscles GLUT4 expression and lipid level in streptozotocin-induced diabetic

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rats. This report indicated that chlorogenic acid may ameliorate the changes of glucose metabolism, insulin sensitivity, glucose 6-phosphatase expression, lipid metabolism and skeletal muscle GLUT4 expression in streptozotocin-induced diabetic rats. The antidiabetic effect of caffeic acid and ferulic acid was also investigated in steptozotocin-induced diabetic mice. [8-9]Caffeic and ferulic acids are naturally occurring hydroxycinnamic acids while chlorogenic acid is family of esters of hydroxycinnamic acids (caffeic, coumaric,sinapic and ferulic acids) with quinic acid.[10]These polyphenols may be used as pharmacologically active analytical markers to characterize or standardize extracts/extract-based products. Therefore, these markers were selected to characterize the active extracts of un-ripe pods of the plant for the first time. The identified markers were used to investigate the molecular interaction of these compounds as ligands with targets proteins.

To provide possible mechanism of action, bioinformatics tools are becoming important to understand the interaction of ligands with different disease-targets.[11])

Among these tools molecular interactions are gaining much attention and these can be studied using the molecular docking approach. Molecular docking can be used to model the interaction between ligand and target protein at atomic level. Docking is not possible with the extracts as these are mixtures of several known and un-known chemical compounds. Such studies are possible by selecting marker compounds which are present in the extract. Several drug-targets could be used to control diabetes but based on the model used to investigate antidiabetic activity, α-amylase, glycogen phosphorelase and hexokinase- I were selected as drug-targets to determine binding affinity of the markers. The results of the present study may be beneficial for finding evidence-based alternative remedy for controlling diabetes.

RESULTS AND DISCUSSION

The results of antidiabetic activity of methanol extract (ME), chloroform extract (CE) and petroleum ether extract (PE) using different models are given in Figure 1.

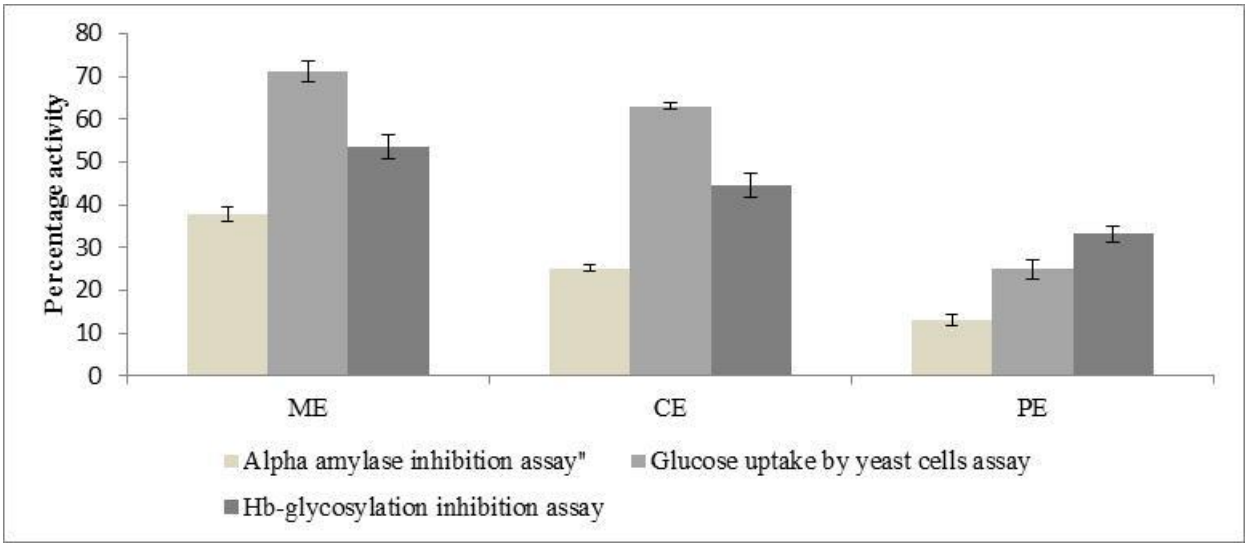

Figure 1. Antidiabetic activity of methanol (ME), chloroform (CE) and petroleum ether (PE) extracts of un-ripened pods of *Vinca rosea* by α-amylase inhibition, glucose uptake by yeast cells and Hb glycosylation inhibition methods

ME, CE and PE of un-ripe pods had shown 37.77, 25.61 and 3.06% activity, respectively, to inhibit α-amylase. The α-amylase is one of the main secretory enzymes of pancreas (about 5–6%) and salivary glands which play a role in the digestion of starch and glycogen. As it is reported by Lo-Piparoet al. [12] that inhibition potential is correlated with the number of hydroxyl groups present in the B ring of polyphenol ligands which form hydrogen bonding between hydroxyl group and catalytic residues of the binding site of enzyme. Therefore, the inhibition of this enzyme is considered a strategy to manage disorders related to carbohydrate uptake such as diabetes, obesity and periodontal diseases. [13]

In the second applied antidiabetic model, the effect of extracts (ME, CE, PE) was observed on the uptake of glucose inside the yeast cells. The ME of un-ripe pods had shown 71.16% uptake of glucose by yeast cells while CE and PE had shown 63.05% and 24.87% uptake of glucose, respectively. The transport of glucose inside the yeast cells is a complicated process which is facilitated by carriers that transport the glucose down the concentration gradient. It means intracellular removal of glucose molecule is necessary to attain the effective transport. [14] If most part of the sugar present inside is converted into metabolites, the internal level of glucose will be down and higher uptake of glucose will be favored. So, there might be possibilities that in the presence of extracts, the uptake of glucose inside the yeast cells took place either by facilitated diffusion and or elevated glucose metabolism.

The extracts of un-ripe pods also have shown significant inhibition of Hb glycosylation. The ME had resulted in to 53.52%, CE with 44.65% and PE with 33.16% inhibition of Hb glycosylation. In glycosylation, glucose reacts non-enzymatically with amino group of Hb through series of reactions resulting into the formation of advance glycated end products (AGEs). The unnecessary accumulation of these AGEs is believed to be responsible for chronic complications of diabetes. [15] The formation of free radicals, reactive carbonyl and dicarbonyl groups take place through series of reaction of glycosylation. Therefore, capturing the free radicals at the early stage of glycosylation leads to decrease the production of reactive carbonyl and dicarbonyl groups which in turn can inhibit the glycated end products. [16] According to Adisa et al. [17] plant products may likely inhibit the formation of AGEs and free radicals in diabetes which implies the reduction of oxidative stress in diabetes. The extracts of un-ripe pods might contain antioxidant phytochemicals which might contribute the anti-glycation effect. The overall results had depicted that ME is an active extract of un-ripe pods of *Vinca rosea* than CE and PE ($p < 0.05$).

Polyphenols-based characterization of ME by HPLC

The analytical markers such as chlorogenic acid, caffeic acid and ferrulic acid were used to characterize ME by HPLC method. The chromatogram of the mixed standards (Figure 2) indicated that peaks of all the compounds were well-resolved.

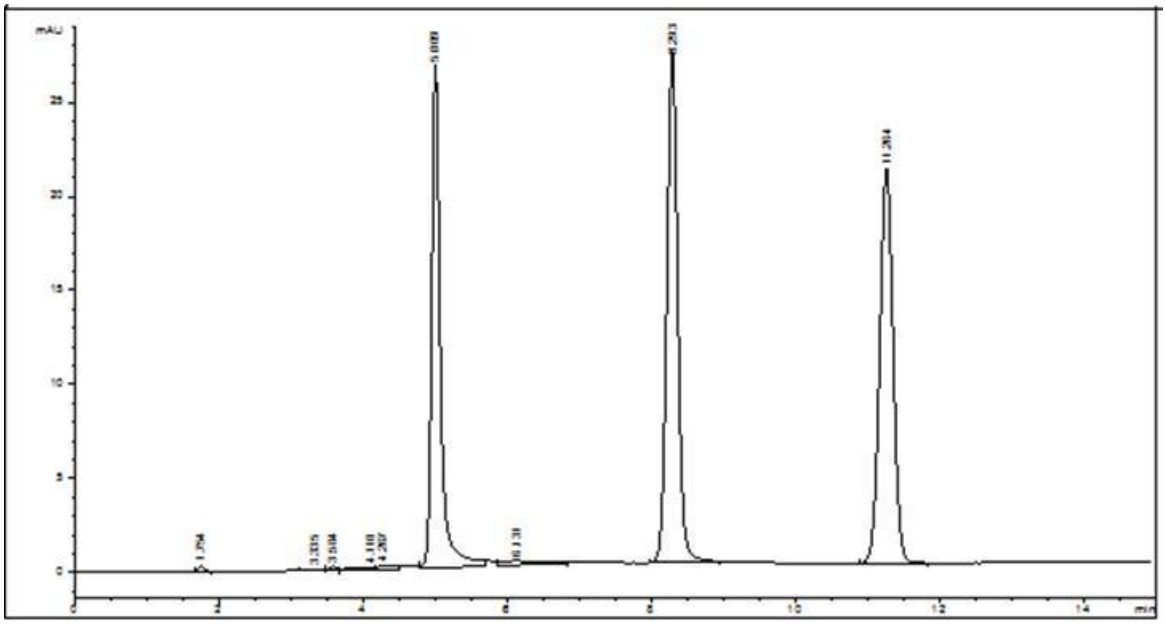

Figure 2. The chromatogram of standards chlorogenic acid (retention time, 5.00), caffeic acid (retention time, 8.29) and ferrulic acid (retention time, 11.264) respectively

Moreover, the peaks of all the standards were Gaussian, hence used to determine system suitability parameters which are given in Table 1.

Table 1. System suitability parameters of the method for the determination of chlorogenic acid, caffeic acid and ferulic acid

Parameters	Chlorogenic acid	Caffeic acid	Ferulic acid	Limit
Theorical plates (N)	17530.47	52335.23	70135.17	> 2000
Capacity factors (k')	5.02	8.28	11.26	≥ 2.0
Tailing factors (As)	1.005	1.00	1.002	≤ 2
Resolution (Rs) of chlorogenic acid and caffeic acid	21.751			≥ 1.5
Resolution (Rs) of caffeic acid and ferulic acid	18.839			≥ 1.5

Theoretical plate (N) is an index that indicates column efficiency
Capacity factor (k') is used to help assess if a peak is going to give reproducible and linear results over time
Tailing factors (As) shows the degree of peak symmetry
Resolution (Rs) is the measure of separation of two peaks of different retention time t

These results show that system is suitable for the analysis of a mix solution of chlorogenic acid, caffeic acid and ferulic acid. The method used for characterization of extract was found to be linear over the whole range investigated with the linear regression equations for chlorogenic acid, caffeic acid and ferulic acid, y = 20.97x +13.96, y =45.56x+16.91, y = 60.2x + 31.32 with correlation coefficient R^2 =0.997, R^2 = 0.996, R^2 = 0.991, respectively. Limit of detection and limit

of quantification for the markers were found to be 0.078572 and 0.238096 µg/mL for chlorogenic acid, 0.023803 and 0.072132 µg/mL for caffeic acid, 0.053463 and 0.162008 µg/mL for ferulic acid. Recovery, intraday and inter-day and accuracy for chlorogenic acid, caffeic acid and ferulic acid were found in a range of 90-110% with relative standard deviation less than 5%.

The chromatogram of ME (Figure 3) indicated two peaks of chlorogenic acid (5.02 min) and caffeic acid (8.29min). The peak areas of these peaks were used to determine contents of chlorogenic acid and caffeic acid using the linear regression equation of chlorogenic acid (y = 20.97x +13.96, R^2 =0.997) and caffeic acid (y =45.56x+16.91, R^2 = 0.996).

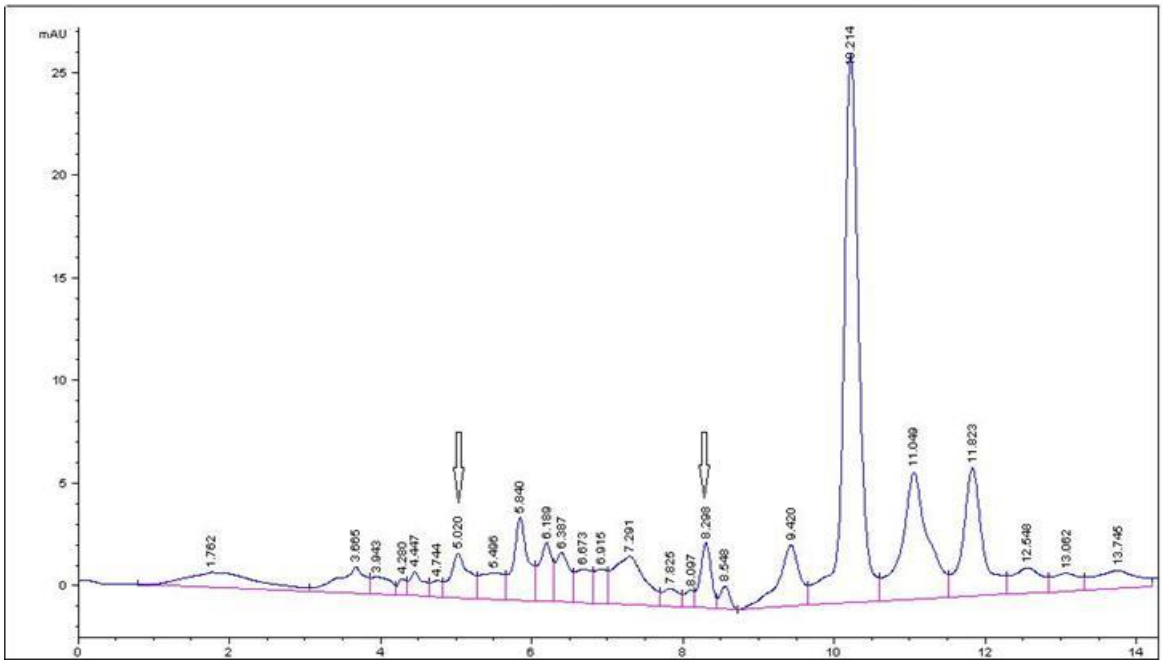

Figure 3. The chromatogram of methanol extract of un-ripened pods of *Vincarosea*showing peaks with retention time 5.02(chlorogenic acid) and 8.29 (caffeic acid) respectively

The amounts of chlorogenic acid and caffeic acid were found to be 0.245 and 0.11 mg/g, respectfully. However, ferulic acid was not detected in ME. Pereira et al. [18] and Nishibe et al. [19] have reported the presence of chlorogenic acid and caffeic acid in the leaves of *Vincarosea*.

Molecular docking studies

The docking studies on the active site of alpha amylase, glycolgenphosphorelase and hexokinase- I were performed by the 1-Click Docking Mcule and UCSF Chimera 1.12 Programs which had shown ligand-target binding modes in terms of lowest docking energy. The target proteins were docked with polyphenols such as chlorogenic acid and caffeic acid, excellent results were seen by lower values of binding energy. The best possible binding mode showing hydrogen bonding of ligands (chlorogenic acid and caffeic acid) with target enzymes are shown in the Figure 4A and 4B; 5A and 5B; 6A and 6B, respectively. Alpha amylase proteins residues ILE235. A, ASP 197.B, ASP 300.A, HIS 305.A have formed four hydrogen bonds with chlorogenicacid. Caffeic acid also has formed one hydrogen bonding with protein residues ARG 195.A. The binding energies and bond distances for both ligands are shown in Table 2.

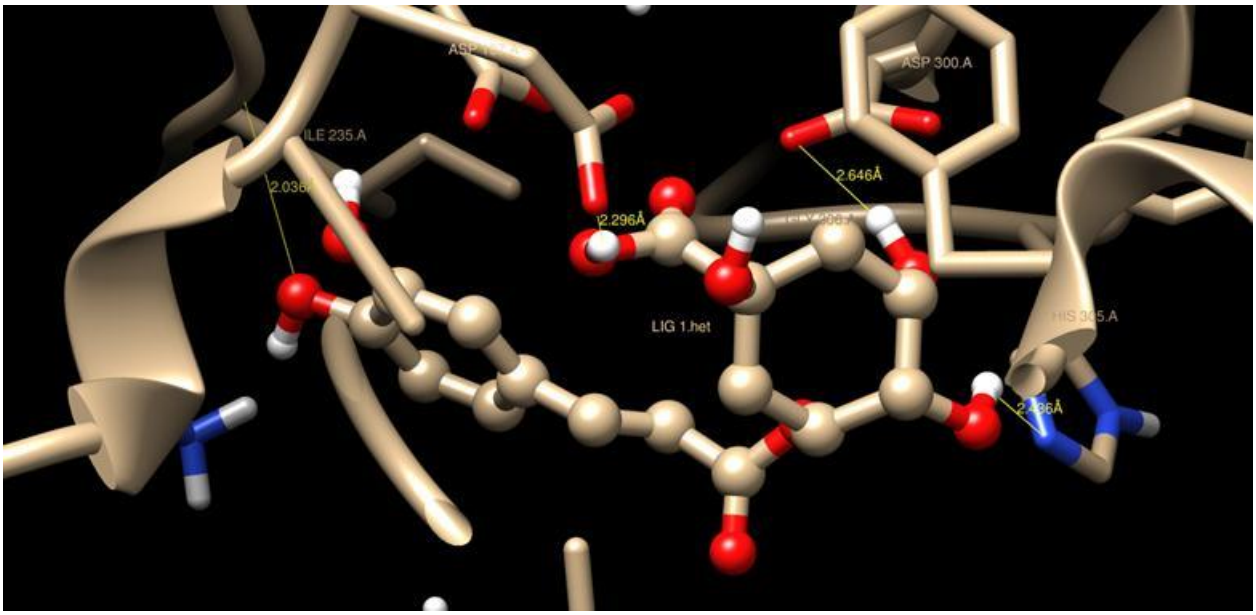

Figure 4A. The predicted structure details of hydrogen bonding of alpha-amylase residues with ligand chlorogenic acid

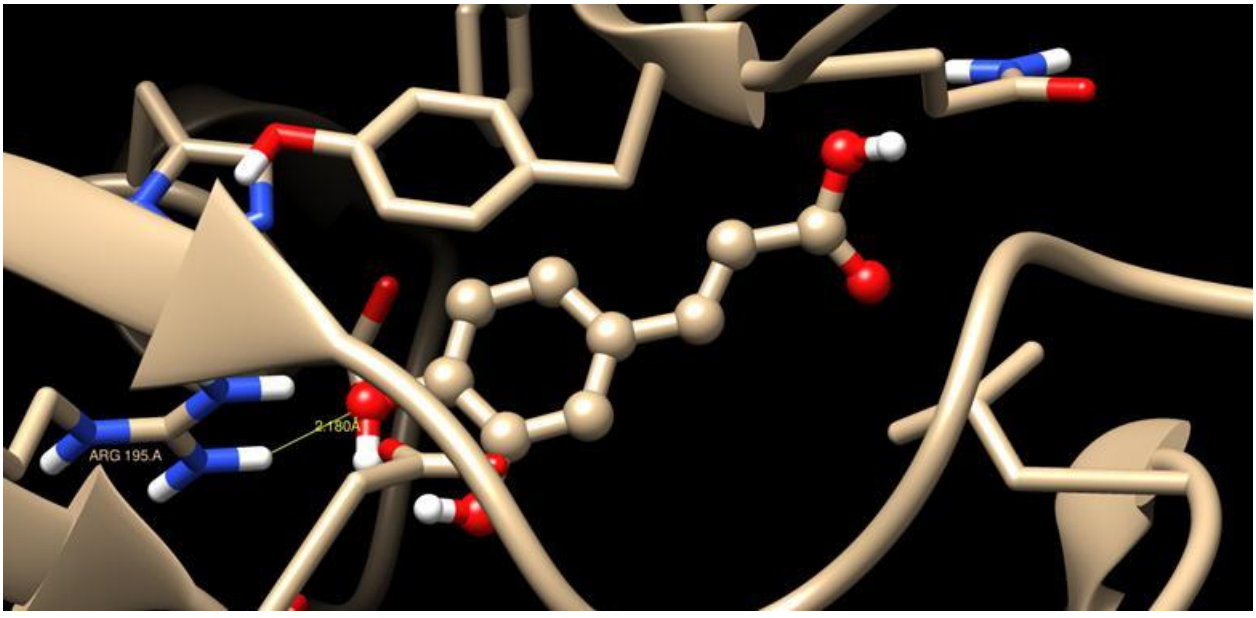

Figure 4B. The predicted structure details of hydrogen bondings of alpha-amylase residues with ligand caffeic acid

Table 2.The docking details of interacting alpha amylase amino acid residues with chlorogenic acid and caffeic acid as ligands

Ligands	Target PDB ID	Organism	Interacting amino acid residues	Binding energy (kcal/mol)	Bond length
Chlorogenic acid	1xcw	Homosapiens	ILE 235. A	-8.1	2.036 Å
			ASP 197.B		2.296 Å
			ASP 300 .A		2.646 Å
			HIS 305 .A		2.436 Å
Caffeic acid	1xcw	Homosapiens	ARG 195.A	-6.6	2.180 Å

The interaction occurs through formation of hydrogen bonding between the hydroxyl groups of the polyphenolsligands and the catalytic residues of the binding site which result in to the formation of bonding for stabilizing the interaction with the active site.

The docking analysis of glycogen phosphorelase (GP) amino acid residues formed eight hydrogen bonds between ARG 77.A, ARG 305.A and ARG 238.A amino acid residues and chlorogenic acid as shown in the Figure 5A, while caffeic acid formed two hydrogen bonds with GLN 67.A and ASP 223.A amino acid residues (Figure 5B).

The least binding energies for chlorogenic acid and caffeic acids are illustrated in the Table 3.

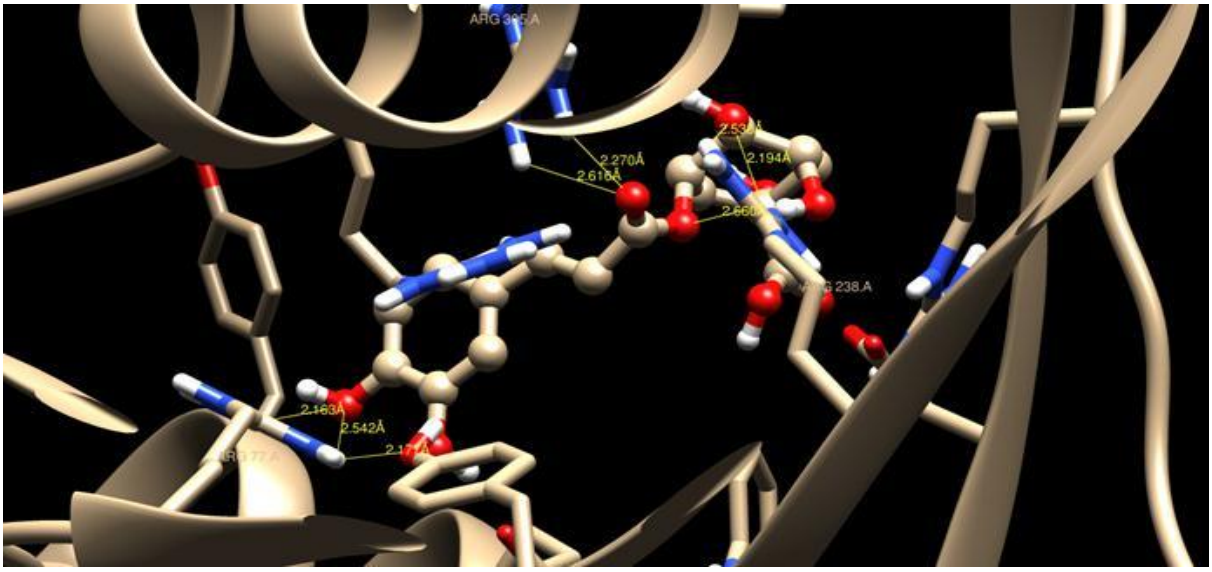

Figure 5A. The predicted structure details of hydrogen bonding of glycogen phosphorelase residues with ligand chlorogenic acid

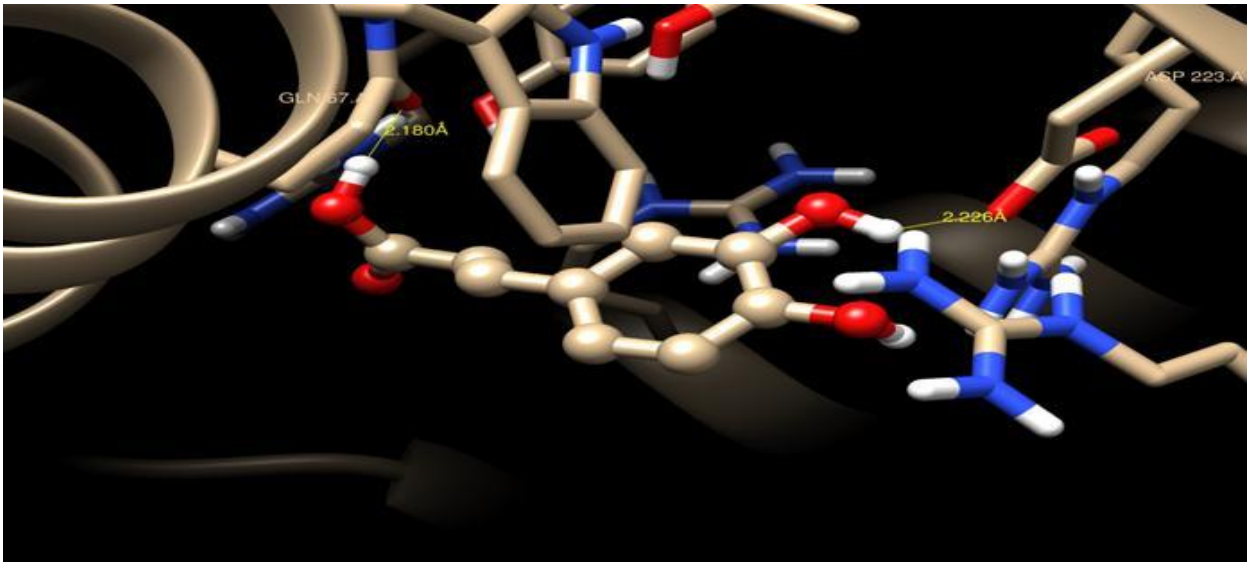

Figure 5B. The predicted structure details of hydrogen bonding of glycogen phosphorelase residues with ligand caffeic acid

Table 3.The docking details of interacting glycogen phosphorelase amino acid residues with chlorogenic acid and caffeic acid as ligands

Ligands	Target PDB ID	Organism	Interacting amino acid residues	Binding Energy (kcal/mol)	Bond length
Chlorogenic acid	1FA9	Homosapiens	ARG 77. A	-7.1	2.163 Å
			ARG 77. A		2.542 Å
			ARG 77.A		2.177 Å
			ARG 305.A		2.616 Å
			ARG 305.A		2.270 Å
			ARG 238.A		2.660 Å
			ARG 238.A		2.194 Å
			ARG 238.A		2.538 Å
Caffeic acid	1FA9	Homosapiens	GLN 67.A	-6.1	2.180 Å
			ASP 223.A		2.223 Å

Inhibition of hepatic glycogen phosphorylase is a promising strategy for treating hyperglycemia in diabetic patient. GP is important target for antidiabetic drugs which catalyzes the breakdown of glycogen to glucose-1-phosphate in liver and tissues. It has two forms, a and b, which are inter convertible. The b form is less active which is transformed into more active a form through phosphorelation.[20] The inhibition of glycogen phosphorelase inhibits the glycogenolysis pathway which in turn reduces the glucose production in liver and lower the glucose level in the blood. [21]

Hexokinase-I is another important enzyme which participate in glycolysis by catalyzing the phosphorelation of glucose into glucose-6-phosphate and provides sufficient energy for the glycolytic process to start. Hexokinase-I allows the muscle cells to take up glucose in the blood and use it as an energy source for different actions. [22]

Therefore the hexokinase was selected as a target to dock with polyphenols to see the binding affinity which may be helpful for the development of plant-based

pharmacophore as hexokinase activator. The docking studies of enzyme hexokinase I has shown a strong binding interaction via formation of three hydrogen bonding with chlorogenic acid (Figure 6A) and three hydrogen bonding with caffeic acid (Figure 6B).

The active binding sites of amino acid residues with bond length and binding energies are given in the Table 4.

The docking studies of chlorogenic acid and caffeic acid with three different target enzymes depicted that they are excellent molecules which docked effectively with the diabetes related targets proteins.

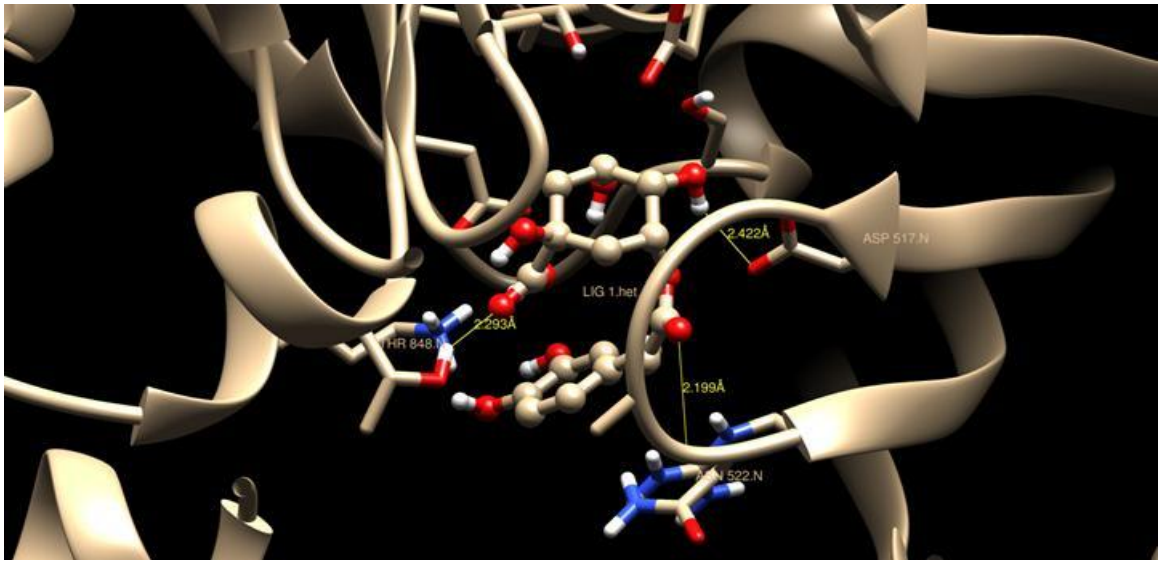

Figure 6A. The predicted structure details of hydrogen bonding of hexokinase-I residues with ligand chlorogenic acid

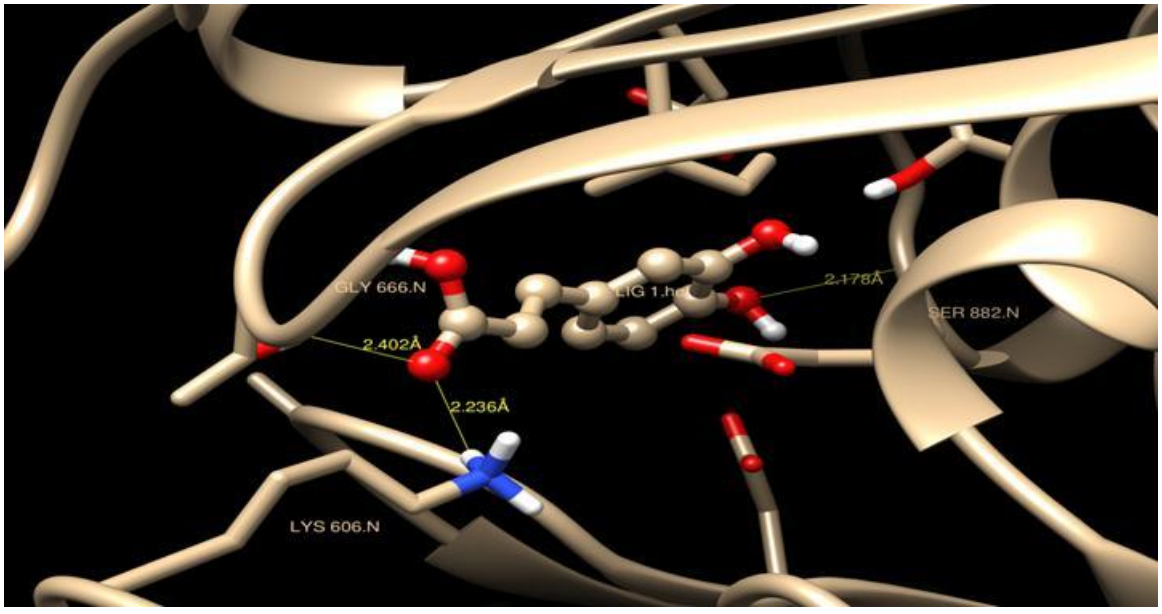

Figure 6B. The predicted structure details of hydrogen bonding of hexokinase-I residues with ligand caffeic acid

Table 4.The docking details of interacting hexokinase-I amino acid residues with chlorogenic acid and caffeic acid as ligands

Ligands	Target PDB ID	Organism	Interacting amino acid residues	Binding Energy (kcal/mol)	Bond length
Chlorogenic acid	1 dgk	Homosapiens	THR 848.N	-7.6	2.293 Å
			ASN 522.N		2.199 Å
			ASP 517.N		2.244 Å
Caffeic acid	1 dgk	Homosapiens	GLY 666.N	-6.4	2.402 Å
			LYS 606.N		2.236 Å
			SER 882.N		2.178 Å

Chlorogenic acid and caffeic acids can play a role in controlling diabetes and diabetes related complications by inhibiting the alpha amylase, glycogen phosphorelase and activating hexokinase.

MATERIALS AND METHODS

Un-ripe pod's collection and extraction

The un-ripe pods of the plant were harvested in the month of October from the Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan and was identified by Dr. Zaheer-UD-Din (Government College University, Lahore, Pakistan) voucher number (GC. Herb. Bot. 2969) deposited at Botany Department, Government College University Lahore, Pakistan. The un-ripe pods were washed, dried under shade and pulverized. Powdered material (15 g) was extracted sequentially by maceration using 50 mL of solvents such as petroleum ether, chloroform and methanol. The material was macerated for 24 h with each of the solvents and procedure was repeated thrice. The extracts were filtered and dried in *vacuo*at 40^{o}C and termed as petroleum ether extract (PE), chloroform extract (CE) and methanol extract (ME).

Chemicals and solvents

The chemicals and solvents used in the present study included metronidazol (Siza International, Lahore, Pakistan), chlorogenic acid, alpha amylase and haemoglobine (China), acarbose (Bayer, Pakistan), methanol, acetonitrile, tetrahydrofuran (RCI Labscan, Thailand), caffeic acid, ferrulic acid, glucose, starch, chloroform, ethanol, acetone, sulphuric acid, FC reagent (Folin- Ciocalteau's), gallic acid, bovine serum albumin, enthrone reagent, nicotinic acid (Sigma Aldrich), n-hexane and acetic acid (E-Merck, Germany).

Software

The 1-Click Docking Mcule and UCSF Chimera 1.12 Software were used for docking and finding hydrogen-bonding affinities.

Antidiabetic activities

Alpha amylase inhibition activity

One milliliter enzyme solution (0.5 mg/mL, in 20 mM phosphate buffer of pH 6.9) and 1 mL of extract/standard (acarbose) having concentration 1 mg/mL were mixed and incubated at 37°C for 10 min. Afterwards, 1 mL of 1% starch solution was added in the reaction mixture and contents were again incubated at 37°C for 10 min. Finally, the reaction was stopped by adding 2 mL of dinitrosalycic acid and heating the mixture in boiling water bath for 8 min. After cooling the contents to room temperature, absorbance was noted at 540 nm [23] and % enzyme inhibition was calculated by the following formula:

$$Percentageactivity = [Absorbanceofcontrol - Absorbanceofsample] / Absorbanceofcontrol$$

Glucose uptake by yeast cells

The method described by Kumar et al. [24] was used to study glucose uptake by yeast cells. Briefly, the yeast cells were rinse with distilled water by centrifugation at 2500 rpm for 5min and procedure was repeated until supernatant

became clear. The yeast cell-pellet thus obtained was suspended in water to prepare suspension (10%, *V/V*). One milliliter of (5mM, 10mM and 20mM) glucose solution and 1mL of each extract/standard (1 mg/mL) were mixed and kept for incubation at 37°C for 10 min. Then, 100µL yeast suspension was mixed and mixture was further incubated for 1h at 37°C. Afterwards, the mixture was centrifuged at 2500 rpm for 5 min and supernatant was used to determine contents of glucose. Metronidazole was taken as a standard. The % of glucose uptake by yeast cells was determined using the following formula:

$$Percentageactivity = [Absorbanceofcontrol - Absorbanceofsample] / Absorbanceofcontrol$$

Non-enzymatic haemoglobin glycosylation inhibition activity

The sample/standard (gallic acid) and all the other solutions were made in phosphate buffer (0.01M) of pH 7.4. One milliliter solution of extract/standard (1 mg/mL), glucose (2%, *W/V*), haemoglobin (0.06%, *W/V*) and gentamycin (0.02%, *W/V*) were mixed and incubated in the dark at room temperature for 72 h. Then, the absorbance was measured at 440 nm.[25]The % inhibition of hemoglobin glycosylation was calculated using the following formula:

$$Percentageactivity = [Absorbanceofcontrol - Absorbanceofsample] / Absorbanceofcontrol$$

Statistical analysis

Statistical analysis of data was done by analyzing variance (ANOVA) and multiple comparison with Bonferoni test (SPSS 12.0). P values < 0.05 were considered significant.

Polyphenol-based characterization of ME by HPLC

The developed method [26] was used for the quantification of chlorogenic acid, caffeic acid and ferulic acid in the methanol extract of un-ripe pods of *Vincarosea*cv. Pink.

Preparation of standards and sample solutions

The stock solutions of chlorogenic, caffeic and ferrulic acids (1 mg/ml) were prepared in mobile phase (acetate buffer: methanol: acetonitrile: tetrahydrofuran in ratio of 65:20:10:5, *V/V/V/V*). Then a mixed standard solution was prepared by mixing 30, 15 and 15µL of stock solution of chlorogenic, caffeic and ferulic acids, respectively and volume was made 1 mL with the mobile phase. The sample solution was made by dissolving the extract in the mobile phase to get a solution having concentration of 5 mg/mL. The standards and sample was filtered using polytetrafluroethylene syringe filter of 0.45µm pore size (Whatmann, Maidstone, England).

System suitability parameters

The system suitability parameters such as number of theoretical plates (N), capacity factor (k′), tailing factor (As) and resolution (Rs) were calculated from the chromatogram obtained analyzing mixed standard solution of chlorogenic acid, caffeic acid and ferulic acid to ensure the accuracy and effectiveness of the system during the analyses.

Validation of HPLC method

The method was validated as per the ICH guidelines [27] for various parameters such as linearity, limit of detection, limit of quantification, precision, accuracy and robustness. For this purpose the different concentrations of chlorogenic, caffeic and ferulic acids (0.15, 0.3, 0.45, 0.9, 1.5, 2.4 and 3.0 µg/mL) were made in mobile phase. For determination of intraday accuracy and precision different concentrations for chlorogenic, caffeic and ferulic acids (0.15 to 0.45 µg/mL) were analyzed six times in the same day. For inter-day accuracy and precision, different concentrations of chlorogenic, caffeic and ferulic acids (0.15 to 0.45 µg/mL) were analysed once daily for six

consecutive days. The amount was calculated from the calibration curves, constructed on each day.

Chromatographic analysis

The analysis was performed by eluting the standards /sample (20 µL) with mobile phase at flow rate of 0.8 mL/min through the column (Agilant 5TC-C_{18} (250X4.6 mm) maintained at 30°C. The detection was carried out at 300 nm and peaks of the samples were identified with the standards. All the standards and sample were analysed in triplicate and contents of the standards in the sample were calculated from the calibration curve. The data acquisition was performed by LC/LCMS software from Windows.

Molecular docking studies

The docking studies of quantified chlorogenic and caffeic acids were performed with the target enzymes (pancreatic α-amylase, glycogen phosphorelase and hexokinase- I using 1-Click docking Mcule software and UCSF Chimera 1.12 Program. The smiles for each chlorogenic acid (C1C(C(C(CC1(C(=O)O)O) OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O) and caffeic acid (C1=CC(=C(C=C1C=CC(=O)O)O)O) were noted from PUB-Chem database. The ligands were docked against the selected protein targets and docking score was noted. UCSF Chimera 1.12 Program was applied on PDB file of each ligands and hydrogen bonding with bond length was determined.

CONCLUSION

The un-ripe pods of *Vinca rosea* cv. Pink have shown significant antidiabetic activity. The active extract, methanol extract, contains chlorogenic acid and caffeic, which have shown good binding affinity with amino acids residues of alpha amylase, glycogen phosphorelase and hexokinase I.

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النشاط المضاد لمرض السكر، والتوصيف القائم على مادة البوليفينول والتفاعل الجزيئي لمستخلص القرون غير الناضجة من فينكا الوردية صنف. لون القرنفل

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ملخص

هدفت الدراسة إلى التعرف على مستخلصات القرون غير الناضجة من نبات الفينكا الوردية. اللون الوردي للنشاط المضاد لمرض السكر والتوصيف القائم على البوليفينول للمستخلصات باستخدام HPLC. علاوة على ذلك ، تم استكشاف التفاعل الجزيئي للعلامات المحددة مع الأهداف المضادة لمرض السكر باستخدام برنامج الالتحام الجزيئي. تم تحضير وفحص مستخلصات مختلفة من القرون غير الناضجة باستخدام نماذج مضادة لمرض السكر مثل امتصاص الجلوكوز بواسطة خلايا الخميرة وتثبيط ألفا أميليز ومقايسات تثبيط الهيموجلوبين بالجليكوزيل. يتميز المستخلص الأكثر نشاطًا باستخدام HPLC باستخدام أحماض الكلوروجينيك والكافيين والفيروليك كواسمات تحليلية. تم أخذ العلامات المحددة كروابط للالتحام الجزيئي مع α-amylase و glycogen phosphorelase و hexokinase–باستخدام 1− Click Docking Mcule Software، وإيجاد روابط ارتباط الهيدروجين بواسطة UCSF Chimera 1.12. أظهرت مستخلصات الميثانول نشاطًا مضادًا لمرض السكر أعلى بنسبة 37.77 و 71.16 و 53.52٪ في تثبيط مقايسة α-amylase وزيادة امتصاص الجلوكوز بواسطة خلايا الخميرة وتثبيط Hb-glycosylation على التوالي. تم العثور على المستخلص يحتوي على 0.25 مجم / جرام من الكلوروجينيك و 0.11 مجم / جرام من أحماض الكافيين. تم العثور على هذه العلامات لتكون روابط جيدة للأهداف المتعلقة بمرض السكري. تشير النتائج إلى أن مستخلص الميثانول له نشاط مضاد لمرض السكر، والذي يمكن تخصيصه لأحماض الكلوروجينيك والكافيين. يمكن استخدام هذه المركبات كواسمات دوائية لتوحيد المستخلصات.

الكلمات الدالة: فينكا الوردية السيرة الذاتية. الوردي، النشاط المضاد لمرض السكر، القرون غير الناضجة، البوليفينول، HPLC عكسي الطور، التفاعل الجزيئي.

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In-vitro Assessment of Essential Oils as Anticancer Therapeutic Agents: A Systematic Literature Review

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ABSTRACT

Cancer is a fatal disease that causes around 9 million deaths annually in developing and developed countries worldwide. Recently, natural products as alternatives for chemical agents have become a growing area of interest. Essential oils (EOs) are secondary metabolites of the plant, with a wide range of bioactivities, such as the anticancer effect. The present systematic review attempts to collect and document the recent studies from 01.01.2016 to 12.31.2020, indicating EOs as anticancer agents in in-vitro studies; data of 144 reports have been extracted. Anticancer effects of 187 distinct EOs on 112 cell lines were summarized; this is a valuable bank for researchers finding proper EO as an anticancer agent. Some EOs having comparable effects with conventional drugs have been suggested. These EOs are good candidates for further studies, such as in-vivo investigations.

Keywords: Systematic review, Essential oil, anticancer activity, in-vitro studies, and food additive.

1. INTRODUCTION

Cancer is a generic name for a large group of incredibly heterogeneous diseases characterized by resisting cell death and abnormal proliferative signaling. The cells may have invasive and metastatic properties to spread to other organs[1]. Cancers are classified based on the tissue type (histological type) or by the body's location (primary site). In histological type, cancer is divided into six main categories: carcinoma (develops from epithelial cells), sarcoma (begin in the bones and in the soft tissues, also called connective), myeloma (a blood cancer), leukemia (cancer of bone marrow and the lymphatic system), lymphoma (develops in lymphocytes), and mixed types [2]. However, the general public recognizes cancer-based on its primary sites (e.g., breast, prostate, lungs, colon, and skin) [2]. The most prevalent cancers are lung, gastric, colon, liver, breast (in women), and prostate (in men), respectively. The incidence of blood, brain, and lymph nodes cancers are highest in children [3]. The biggest risk factor for cancer is advancing age. For example, about 75% of men develop prostate cancer by 75 years[4]. The environment, cigarette smoke, diet, infectious diseases, ionizing, and non-ionizing radiation are the other most important causes of cancer, respectively [5].

Cancer is caused by a dysfunction in multiple systems, including signaling and biochemical networks of normal cells, and is a very complex genetic, epigenetic, and environmental disease with a wide variety of tissue, tumor, and cellular levels, and this diversity can lead to the failure

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of conventional therapies [6]. Over time, the accumulation of mutations and epigenetic changes in the cell alters biochemical networks and signal conduction. A combination of these changes eventually leads to cell transformation and cancer [7]. Surgery, radiation therapy, and chemotherapy are effective methods to treat cancer disease. Due to cancer's high proliferative capacity and tumorigenicity, cell proliferation inhibition and inducing apoptosis are effective anti-***tumor therapeutic strategies***. Thus, chemotherapy is used as a primary approach in cancer treatment after surgery [8]. Chemotherapy is mainly used to treat disseminated tumors such as breast, prostate, and colorectal cancer. Despite its high effectiveness, long-term use of chemotherapy can lead to several side effects as well as drug resistance [9]. Common side effects of chemotherapy include hair loss, nausea, and emesis [10, 11]. In addition, drug-resistance is a significant factor of failure in chemotherapy. Drug resistance includes primary resistance starting before chemotherapeutic and acquired resistance after chemotherapeutic exposure [12].

Regarding the background and disadvantage of chemotherapy, essential oils (EOs) as anticancer therapeutic agents are being widely explored in recent years. EOs are natural oils secreted as secondary metabolites in aromatic plants [13, 14]. They possess many biological properties, such as larvicidal effect [15], antifungal/bacterial effect [16, 17], leishmanicidal effect [18, 19], and anticancer activity [20, 21]. Besides, aromatherapy originated from traditional medicine, using herbal materials such as EOs to treat or prevent the diseases. In recent years, EOs have been introduced as an alternative to bypass the well-known side effects caused by synthetic chemotherapeutic drugs[22, 23]. Interestingly, phytochemicals are generally inexpensive and have selective cytotoxic effects on cancer cells with minimum influences on healthy cells [24, 25]. Also, plant-derived chemical compounds have been reported to prevent carcinogenesis processes by cellular arrest, inducing both the intrinsic and extrinsic apoptosis pathways, inhibiting the mutagen entering the cell, and reducing oxidative stress in cells [26, 27].

Numerous reports could be found about evaluating anticancer activities of EOs against different cell lines in the literature. However, we could not find any systematic review that reviews the efficacy of EOs as anticancer agents compared to chemical drugs. Therefore, this study has considered the newest reports about using EOs as anticancer agents from 01.01.2016 to 12.31.2020.

2. Method

2.1. Data resource

Numerous reports have been published on the anticancer effect of EOs; thus, data resource was excluded to PubMed, as the main source for medical research. It (https://www.ncbi.nlm.nih.gov/pubmed/advanced) was searched from 01.01.2016 to 12.31.2020, using special keywords; 547 papers were obtained at this stage (see Table 1).

Table 1: Steps for data gathering

Steps No.	Query	Results
1	essential oil*[Title/Abstract] Filters: from 2016/1/1 - 2020/12/31	7233
2	cancer*[Title/Abstract] Filters: from 2016/1/1 - 2020/12/31 "cancer*"[Title/Abstract]	611308
3	tumor*[Title/Abstract] Filters: from 2016/1/1 - 2020/12/31 "tumor*"[Title/Abstract]	398845
4	Step 3 or 2	786637
5	Step 1 and Step 4	547

2.2. Exclusion criteria

Abstracts of the 547 papers were studied; papers that only evaluated the anticancer activity of ingredients of EOs without investigating the total EO were excluded. After that, all documents reported in-vitro studies, formulated forms of EOs, and review studies were excluded. Full texts of remaining studies were then collected to extract required information, including names of plants, cell lines names, exposure time of EOs with cell lines, and EO efficacy (i.e., Inhibitory concentration 50% (IC_{50})). Eventually, articles that did not contain the mentioned information were also excluded. In total, data of 144 papers were extracted; names of 112 mentioned cell lines are listed in Table 2.

Table 2: Cancer cell lines names

Code	Cell line name	Code	Cell line name
2008	Human ovarian cancer	LoVo	Human colon carcinoma
22RV1	Human prostate cancer	LS174D3	Human colon cancer
4T1	Mouse mammary tumor	LU134AM	Human small-cell lung cancer
A2058	Human melanoma cancer	LU135	Human small-cell lung cancer
A2780	Human ovarian cancer	LU165	Human small-cell lung cancer
A375	Human melanoma cancer	M059J	Human glioblastoma
A431	Human cervical carcinoma	MCF102A	Immortalized normal breast epithelial
A549	Human lung cancer	MCF10A	Human breast fibrocystic epithelial cells
A549CS	Human lung adenocarcinoma	MCF7	Human breast adenocarcinoma
ACP03	Human gastric cancer	MCF7/ADR	Resistant human breast cancer
AGP01	Human gastric cancer	MDA-MB231	Human breast adenocarcinoma
AGS	Human stomach cancer	MDA-MB468	Human breast cancer
B164A5	Mouse melanoma	MIA-PaCa2	Human pancreatic carcinoma
B16F10	Mouse melanotic cancer	MKN45	Human gastric adenocarcinoma
B16F10Nex2	Murine melanoma	MN1112	Human small-cell lung cancer
BEAS2B	Human normal lung	MV3	Human Melanoma
BxPC3	Human pancreatic carcinoma	MV411	Human leukemia
C26	Mouse colon carcinoma	Mz-ChA1	Human extrahepatic cholangiocarcinoma
Caco2	Human colorectal adenocarcinoma	NCCIT	Human embryonal carcinoma cancer cell
CAL27	Human oral squamous cell carcinoma	NCI/ADR-RES	Human ovarian tumor
CCRF-CEM	Human T lymphoblast leukemia	NCI-H1975	Human non-small cell lung adenocarcinoma
CEM/ADR5000	Human adriamycin resistant leukemia	NCI-H460	Human lung cancer

Code	Cell line name	Code	Cell line name
Colo205	Human colon cancer	NIH3T3	Mouse embryonic non-tumor fibroblast
DU145	Human prostate cancer	OV2008	Human ovarian cancer
EFO21	Human ovary cystadenocarcinoma	OVCAR3	Human ovarian cancer
FaDu	Human squamous cell carcinoma of the pharynx	P815	Murine mastocytoma
FM94	Human melanoma	Panc1	Human pancreatic carcinoma
FTC133	Human follicular thyroid carcinoma	Panc28	Human pancreatic adenocarcinoma
H157	Human oral squamous cell carcinoma	PC2	Human prostate carcinoma
H1975	Human lung cancer	PC3	Human prostate cancer
HA22T/VGH	Human hepatocellular carcinoma	PCO3	Human prostate cancer
HaCat	Human keratinocytes	PDL	Human periodontal ligament fibroblasts
HCT116	Human colorectal carcinoma	PSN1	Human pancreatic cancer
HEK293	Human embryonic normal kidney fibroblast	Raji	Human lymphoblastoid cells
HeLa	Human cervical carcinoma	SCC25	Human squamous cell carcinoma of the tongue
HeLa	Human cervical cancer	SCC4	Human squamous cell carcinoma
HelaR2	Human cervical carcinoma	SF763	Human glioblastoma
Hep2	Human epidermoid cancer	SF767	Human glioblastoma
Hep3B	Human liver cancer	SH-SY5Y	Human neuroblastoma
Hepa1c1c7	Murine hepatoma	SKBR3	Human breast adenocarcinoma
HepG2	Human hepatocellular carcinoma	SKHep1	Human liver cancer
HL60	Human promyelocytic leukemia	SKMEL19	Human melanoma cancer
HL60R	Human acute myeloid leukemia multidrug-resistant	SKOV3	Human ovarian cancer
HOC/DOX,A2780/ADR	Doxorubicin-resistant human ovarian carcinoma	SUM149	Human breast cancer cell
HOC-A2780	Human ovarian carcinoma	SW620	Human colon cancer
HSC3	Human oral squamous cell carcinoma	T24	Human transitional cell carcinoma
HT1080	Human fibrosarcoma	T47D	Human epithelial breast cancer
HT29	Human colon adenocarcinoma	T75	Human fibroblast

Code	Cell line name	Code	Cell line name
HUVEC	human umbilical vein endothelial cell	THP1	Human acute monocytic leukemia
Jurkat	Human acute T lymphocytic leukemia	U251	Human glioblastoma
K562	Human Chronic myelogenous leukemia	U26684	Human multiple myeloma
KB	Human oral epidermoid carcinoma	U87MG	Human glioblastoma
KBM5	Human chronic myeloid leukemia	U937	Human leukemia
KON	Human oral carcinoma	UCT-Mel1	Human melanoma
LIM1215	human colon cancer	VERO	Animal normal kidney fibroblast
LNCaP	Human prostate carcinoma	Y79	Human eye cancer

3. Documentation of the anticancer properties of EOs

In 144 reviewed reports, anticancer effects of 61 EOs were investigated compared to commercial drugs (see Table 3). Other documents have been categorized as their examined cell lines as follows. Anticancer effects of 91 EOs were investigated on some cell lines from different organs (see Table 4). Anticancer effects of 19 were investigated on the digestive system associate cell lines (see Table 5). Anticancer effects of 19 were investigated on genital organs associate cell lines (see Table 6. Anticancer effects of 19 were investigated on breast, lung, and skin cell lines (see Table 7).

From Table 3, differences between IC_{50}s of EOs against different cell lines are substantial. For instance, IC_{50} of *Eryngium campestre* against A375 and HCT116 and IC_{50} of *Eryngium amethystinum* against HCT116 are around 1µg/mL [25]**,** while IC_{50} of *Mentha spicata* EO is 710 µg/mL against THP1 [26]. Alternatively, even *Glycyrrhiza triphylla* EO against U87MG, MDA-MB231, SKBR3, 4T1, and NIH3T3 were reported as inactive [27]. Moreover, EOs show a selective effect on different cell lines. For example, IC_{50} of *Lippia citriodora* EO against A375 and THP1 is 9.10 and 111.00 µg/mL, respectively [28]. Reported IC_{50} values for *Myrcia splendens* EO against A549, MCF7, and HaCat are 100.99, 5.59, and 21.58 µg/mL, respectively [29]. Also, 165.00 and 32.00 µg/mL are reported IC_{50} values of *Foeniculum vulgare* EO against CEM/ADR 5000 and CCRF-CEM, respectively [30].

Comparing IC_{50} of different EOs with commercial drugs against defined cell lines are fascinating: in some cases (underlined in Table 2), their potency is comparable with the drugs. For instance, IC_{50} of *Eryngium campestre* EO and cisplatin against three cell lines, including A375, MDA-MB231, and HCT116, are close together (around 2 µg/mL) [25]. IC_{50} of *Myrcia splendens* EO against MCF7 is comparable with doxorubicin (i.e., 5.59 and 2.10 µg/mL, respectively) [29]. Effectiveness of *Lippia alba* (IC_{50}: 63.98 µg/mL) against A549 is higher than paclitaxel (IC_{50}: 84.30 µg/mL) [31]. However, in most cases, the IC_{50} value of EO is substantially larger than commercial drugs.

Table 3: Researches comparing EOs with commercial drugs

Ref	Plant name	Exp. Time	Cell lines	IC_{50} (µg/mL)	
				EO	Drug
[29]	*Myrcia splendens*	48h			**Doxorubicin**
			A549	100.99	0.90
			MCF7	5.59	2.10
			HaCat	21.58	0.40
[32]	*Thymus alternans*	72h			**Cisplatin**
			A375	5.51	0.43
			MDA-MB231	5.96	2.94
			HCT116	8.45	2.42
[25]	*Eryngium campestre*	72h			**Cisplatin**
			A375	1.57	0.41
			MDA-MB231	2.99	2.74
			HCT116	1.64	2.34
[25]	*Eryngium amethystinum*	72h			**Cisplatin**
			A375	2.78	0.41
			MDA-MB231	5.32	2.74
			HCT116	1.65	2.34
[33]	*Zanthoxylum monogynum*	18h			**Cisplatin**
			B16F10	60.00	52.80
			A2058	34.00	43.10
			MCF7	65.70	ND
			HeLa	62.00	20.30
			HL60	11.00	20.90
			T75	60.00	ND
[34]	*Schizogyne sericea*	72h			**Cisplatin**
			A375	3.50	0.40
			MDA-MB231	6.60	2.90
			HCT116	3.40	2.40
[23]	*Cymbopogon citratus*	72h			**Cisplatin**
			LNCaP	6.40	2.90
			PC3	32.10	11.20
			SF767	45.10	0.40
			SF763	172.10	8.20

Ref	Plant name	Exp. Time	Cell lines	IC_{50} (µg/mL)		
					EO	Drug
[31]	*Lippia alba*	18h			**Cisplatin**	**Paclitaxel**
			B16F10Nex2	45.82	52.8	ND
			A549	63.98	ND	84.30
			MCF7	>100	ND	171.50
			HUVEC	>100	52.80	ND
[28]	*Lippia citriodora*	72h			**Etoposide**	
			A375	9.10	ND	
			HepG2	74.00	0.60	
			MCF7	89.00	1.67	
			Caco2	71.00	7.30	
			THP1	111.00	0.45	
[35]	*Ferulago trifida*	72h			**Tamoxifen**	
			MCF7	22.00	3.60	
			A549	25.00	10.70	
			HT29	42.55	2.50	
[30]	*Foeniculum vulgare*	24h			**Doxorubicin**	
			HeLa	207.00	4.50	
			Caco2	75.00	1.10	
			MCF7	59.00	1.30	
[30]	*Foeniculum vulgare*	48h	CEM/ADR 5000	165.00	1.40	
			CCRF-CEM	32.00	0.25	
[27]	*Glycyrrhiza triphylla*	24h			**Doxorubicin**	
			U87MG	NA	0.46	
			MDA-MB231	NA	0.16	
			C26	400	0.15	
			SKBR3	NA	0.79	
			4T1	NA	0.26	
			NIH3T3	NA	0.37	
[36]	*Ajuga chamaepitys*	72h			**Cisplatin**	
			A375	67.44	0.44	
			MDA-MB231	59.24	2.04	
			HCT116	64.12	2.65	
[37]	*Sideritis montana*	72h			**Cisplatin**	
			A375	34.89	0.45	
			MDA-MB231	32.32	2.92	
			HCT116	31.84	2.39	

Ref	Plant name	Exp. Time	Cell lines	IC_{50} (µg/mL)	
				EO	Drug
[38]	*Cyperus longus*	48h			**Paclitaxel**
			PC3	22.25	0.09
			MCF7	12.55	3.45
[26]	*Ocimum basilicum*	72h			**Etoposide**
			HepG2	180.00	0.65
			Caco2	71.00	7.30
			MCF7	170.00	1.67
			THP1	670.00	0.45
[26]	*Mentha spicata*	72h			**Etoposide**
			HepG2	220.00	0.65
			Caco2	162.00	7.30
			MCF7	284.00	1.67
			THP1	710.00	0.45
[26]	*Pimpinella anisum*	72h			**Etoposide**
			HepG2	390.00	0.65
			Caco2	250.00	7.30
			MCF7	300.00	1.67
			THP1	110.00	0.45
[26]	*Fortunella margarita*	72h			**Etoposide**
			HepG2	ND	0.65
			Caco2	100.00	7.30
			MCF7	ND	1.67
			THP1	100.00	0.45
[39]	*Thymus munbyanus*	72h			**Cisplatin**
			A375	46.95	0.40
			MDA-MB231	97.27	2.29
			T98G	51.54	2.22
[40]	*Eugenia uniflor*	24h			**Doxorubicin**
			MCF7	76.40	29.83
[41]	*Lippia citriodora*	48h			**Methotrexate**
			P815	7.75	2.50
[42]	*Rosa damascene*	48h			**Cisplatin**
			A549	36.43	8.06
			NIH3T3	42.93	16.67

Ref	Plant name	Exp. Time	Cell lines		IC_{50} (µg/mL)	
					EO	Drug
43	*Cyphostemma juttae*	24h			**N-acetyl-L-cysteine (NAC)**	
					73.60	
			MDA-MB231	46.00	98.60	
			SUM 149	64.00		
44	*Erythrina corallodendron*	24h			**Doxorubicin**	**Capecitabine**
			MDA-MB231	3.44	0.56	1.09
			MCF7	4.91	0.86	1.47
45	*lemongrass*	72h			**Doxorubicin**	
			HOC-A2780	55.20	0.02	
			HOCDOX, A2780ADR	197.80	2.86	
46	*Psidium guajava*	24h			**Doxorubicin**	
			MCF7	96.80	62.10	
			HeLa	128.70	5.30	
			M059J	103.60	16.20	
47	*Cannabis sativa*	24h			**Doxorubicin**	
			MCF7	83.20	7.60	
			Caco2	28.70	23.30	
			Mz-ChA1	22.30	15.70	
48	*Conobea scoparioides*	72h			**Doxorubicin**	
			MCF7	45.52	0.22	
			HepG2	41.86	0.04	
			HCT116	13.50	0.08	
49	*Tamarix aphylla*				**Doxorubicin,**	**Cisplatin**
			MCF7	26.65	0.01	1.17
			Caco2	130.55	0.10	1.11
			Panc1	88.74	0.06	5.97
50	*Croton matourensis Aubl*	72h			**Doxorubicin**	
			MCF7	23.30	0.30	
			HCT116	28.90	0.10	
			HepG2	28.50	0.03	
			HL60	17.80	0.04	
51	*Plectranthus cylindraceus*	48h			**Dasatamib**	
			HeLa	3.97	5.57	
			HepG2	3.88	4.05	
			HT29	3.91	5.24	

Ref	Plant name	Exp. Time	Cell lines	IC_{50} (µg/mL)	
				EO	Drug
[51]	*Plectranthus asirensis*	48h			**Dasatamib**
			HeLa	7.51	5.57
			HepG2	7.19	4.05
			HT29	6.82	5.24
[51]	*Plectranthus barbatus*	48h			**Dasatamib**
			HeLa	4.97	5.57
			HepG2	4.99	4.05
			HT29	4.93	5.24
[52]	*Guatteria megalophylla Diels*	72h			**Doxorubicin**
			HL60	12.51	0.02
			MCF7	35.45	6.16
			CAL27	7.58	1.09
			HSC3	14.90	0.86
			HepG2	21.62	0.02
			HCT116	30.27	0.02
[53]	*Scrophularia Atropatana*	48h			**Methotrexate**
			MCF7	60.70	0.16
[54]	*Cyperus articulatus*	24h			**Doxorubicin**
			HepG2	28.50	0.03
			HCT116	>50	0.10
			MCF7	36.70	0.30
			HL60	33.51	0.04
			B16F10	39.70	0.20
[55]	*Thymus bovei Benth*	24h			**Cisplatin**
			HeLaR2	7.22	4.24
			LS174D3	9.30	5.21
			A549C5	8.62	5.43
[56]	*Isodon rugosus*	24h			**Doxorubicin**
			HepG2	69.20	80.00
[57]	*Mentha citrata*	48h			**Doxorubicin**
			HCT116	80.60	37.60
[58]	*Myrrh*	24h			**Doxorubicin**
			HepG2	41.52	9.79
			MCF7	10.93	4.25
			HCT116	19.71	7.22
[59]	*Lavandin*	72h			**Puromycin**
			HL60	111.00	**0.57**

Ref	Plant name	Exp. Time	Cell lines	IC_{50} (µg/mL)	
				EO	Drug
[60]	*Tarchonanthus*	24h			**Doxorubicin**
	Camphoratus		MCF7	12.50	1.20
			HepG2	38.00	1.30
			A549	50.00	1.10
[61]	*Lemon oil*	72h			**5-fluorouracil**
			U87MG	440.10	464.20
			MKN45	220.90	271.10
			A431	62.80	5.20
[61]	*Cardamom oil*	72h			**5-fluorouracil**
			U87MG	NA	464.20
			MKN45	NA	271.10
			A431	166.30	5.20
[61]	*Jasmine oil*	72h			**5-fluorouracil**
			U87MG	336.20	464.20
			MKN45	275.00	271.10
			A431	99.80	5.20
[62]	*Zingiber zerumbet*	72h			**Cisplatin**
			A549	14.51	1.91
			PC3	11.23	2.16
			K562	10.48	5.10
[63]	*Satureja thymbra*	48h			**Doxorubicin**
			MCF7	2.75	3.45
			HCT116	2.45	0.40
[64]	*Artemisia judaica*	-*			**Vinblastine sulfate**
			MCF7	28.51	ND
			Jurkat	63.71	0.10
			T24	171.13	63.31
			HT29	73.01	21.40
			HEK293	>300.00	51.50
			HeLa	54.13	2.50
[64]	*Artemisia monosperma*	-*			**Vinblastine sulfate**
			MCF7	15.15	ND
			Jurkat	11.00	0.10
			T24	119.00	63.31
			HT29	10.10	21.40
			HEK293	>300.00	51.50
			HeLa	9.10	2.50

Ref	Plant name	Exp. Time	Cell lines	IC_{50} (µg/mL)	
				EO	Drug
[64]	*Callistemon viminals*	-*			**Vinblastine sulfate**
			MCF7	25.15	ND
			Jurkat	53.10	0.10
			T24	166.15	63.31
			HT29	10.51	21.40
			HEK293	>300.00	51.50
			HeLa	18.75	2.50
[64]	*Citrus aurantifolia*	-*			**Vinblastine sulfate**
			MCF7	11.11	ND
			Jurkat	17.10	0.10
			T24	>300.00	63.31
			HT29	230.84	21.40
			HEK293	>300.00	51.50
			HeLa	58.75	2.50
[64]	*Citrus limon*	-*			**Vinblastine sulfate**
			MCF7	9.52	ND
			Jurkat	15.34	0.10
			T24	216.70	63.31
			HT29	231.91	21.40
			HEK293	>300.00	51.50
			HeLa	51.04	2.50
[64]	*Citrus paradisi*	-*			**Vinblastine sulfate**
			MCF7	8.10	ND
			Jurkat	14.52	0.10
			T24	113.60	63.31
			HT29	220.00	21.40
			HEK293	>300.00	51.50
			HeLa	46.15	2.50
[64]	*Cupressus macrocarpa*	-*			**Vinblastine sulfate**
			MCF7	25.40	ND
			Jurkat	30.54	0.10
			T24	>300.00	63.31
			HT29	124.80	21.40
			HEK293	>300.00	51.50
			HeLa	24.16	2.50

Ref	Plant name	Exp. Time	Cell lines	IC_{50} (µg/mL)	
				EO	Drug
[64]	*Origanum vulgare*	-*			**Vinblastine sulfate**
			MCF7	8.11	ND
			Jurkat	27.05	0.10
			T24	105.50	63.31
			HT29	12.18	21.40
			HEK293	>300.00	51.50
			HeLa	13.41	2.50
[64]	*Pelargonium graveolens*	-*			**Vinblastine sulfate**
			MCF7	61.00	ND
			Jurkat	178.50	0.10
			T24	270.13	63.31
			HT29	195.33	21.40
			HEK293	>300.00	51.50
			HeLa	51.24	2.50
[64]	*Rosmarinus officinalis*	-*			**Vinblastine sulfate**
			MCF7	36.50	ND
			Jurkat	73.11	0.10
			T24	118.31	63.31
			HT29	18.17	21.40
			HEK293	>300.00	51.50
			HeLa	27.25	2.50
[64]	*Schinus molle*	-*			**Vinblastine sulfate**
			MCF7	41.33	ND
			Jurkat	14.85	0.10
			T24	>300.00	63.31
			HT29	18.35	21.40
			HEK293	>300.00	51.50
			HeLa	119.50	2.50
[64]	*Thuja occidentalis*	-*			**Vinblastine sulfate**
			MCF7	57.35	ND
			Jurkat	95.52	0.10
			T24	>300.00	63.31
			HT29	125.50	21.40
			HEK293	>300.00	51.50
			HeLa	22.50	2.50

*Exposure time has not been reported.

In Table 4, the anticancer activity of 91 EOs against different cancer cell lines is demonstrated. Since these reports, positive controls were not considered; reviewing their efficacy against cancer cell lines is not expected to be precise for us. Thus, their potencies are compared with drugs in Table 3 when having similar same exposure times. For instance, IC_{50} of *Pinus eldarica* and *Pallines spinosa* EOs, with an exposure time of 48h, against MCF7, are 0.03 and 0.25 µg/mL, respectively [65, 66]. This value for doxorubicin and paclitaxel is 2.10 and 3.45 µg/mL, respectively [29, 38]. Reported IC_{50} values for EOs of *Pallenis spinosa*, *Oliveria decumbens*, and doxorubicin with an exposure time of 24h against MCF7 is 0.50, 0.06, and 1.30 µg/mL, respectively [30, 67, 68].

Interestingly, IC_{50} of EO of *Pinus spinosa* exposed 24h against different cancer cell lines including HL60, K562, Jurkat, HepG2, HT1080, and Caco2 is almost under 1 µg/mL [67]. Furthermore, its IC_{50} against other cancer cell lines with an exposure time of 48h is under 1 µg/mL [67]. IC_{50} (24h) of *Lavandula stoechas* against different cell lines were reported as MV3 0.01, MDA-MB231 0.25, and AGS 0.03 µg/mL [69]. Also, IC_{50} of *Pinus eldarica* with an exposure time of 48h against HeLa is 0.03 µg/mL [65]. Others EOs with IC_{50} of <10 µg/mL include *Anacamptis coriophora* (IC_{50} against 2008 and BxPC3: 6.90 and 3.30 µg/mL, respectively) [70], *Foeniculum vulgare* (IC_{50} against MDA-Mb and HeLa: 0.68 and 1.26 µg/mL, respectively) [71], *Aloysia citriodora* (IC_{50} against P815: 6.60 µg/mL) [72], *Cinnamomum glanduliferum* (IC_{50} against HCT116: 9.10 µg/mL) [73] and *Anaxagorea brevipes* (IC_{50} against: PC3 (9.6 µg/mL) [74]. Beside potency (IC_{50}) of *Sideritis raeseri* with exposure time of 72h were excellent; A375: 0.15, PC2: 0.21, and Caco 2: 0.17 µg/mL [75]. On the other hand, the potencies of many other EOs are considerably higher than commercial drugs.

Table 4: Researches that targeted cell lines of more than one organ type

Ref.	Plant name	Exp. Time	Cell lines and related IC_{50} (µg/mL)		
65	*Pinus eldarica*	48h	HeLa: 0.03	MCF7: 0.03	
66	*Pallenis spinosa*	24h	HL60: 0.25	HepG2: 0.71	Jurkat: 0.42
			K562: 0.66	HT1080: 1.22	MCF7: 0.50
			Caco2: 2.35		
67	*Pallines spinosa*	48h	MV411: 0.09	U937: 0.33	K562: 0.18
			MCF102A: 1.30	MCF7: 0.25	Jurkat: 0.22
			MDA-MB231: 0.21	HL60: 0.13	THP1: 0.24
70	*Anacamptis coriophora*	72h	2008: 6.90	BxPC3: 3.30	
72	*Aloysia citriodora*	48h	P815: 6.60	VERO: 32.90	MCF7: 34.72
76	*Foeniculum vulgare*	-h	MDA-Mb: 0.68	HeLa: 1.26	
73	*Cinnamomum glanduliferum*	24h	HCT116: 9.10	MCF7: 57.30	HepG2: 42.40
74	*Anaxagorea brevipes*	-h	MCF7: 12.80	PC3: 9.60	NCI-H460: 13.00
77	*Ballota undulate*	48h	HepG2: 54.75	MCF7: > 100	
77	*Ballota saxatilis*	48h	HepG2: 65.41	MCF7: > 100	
77	*Ballota nigra*	48h	HepG2: 69.92	MCF7: > 100	
8	*Navel orange*	24h	A549: 17.53	22RV1: 45.74	
78	*Baccharis milleflor*	24h	Jurkat: 42.91	HL60: 23.06	Raji: 39.15

Ref.	Plant name	Exp. Time	Cell lines and related IC_{50} (µg/mL)		
79	*Kelussia odoratissima*	48h	MDAMB468: 85.00	Y79: 82.00	SKOV3: 120.00
				A549: 145.00	K562: 70.00
80	*Rosmarinus officinalis*	48h	HeLa: 11.00	MCF7: 253.00	
81	*Pistacia lentiscus*	24h	FTC133: 376.00	LNCaP: 616.00	HepG2: 512.00
			NCI-H1975: 400.00	HeLa: 520.00	CaCo2: 640.00
			MDA-MB231: 616		
82	*Pinus heldreichii*	24h	HeLa: 200.00	MCF7: 1000.00	CaCo2: 200.00
82	*Pinus Peuce*	24h	HeLa: 70.00	MCF7: 600.00	CaCo2: 200.00
82	*Pinus Mugo*	24h	HeLa: 3000.00	MCF7: 3000.00	CaCo2: 200.00
83	*Hedychium spicatum*	-h	DLD1: 42.00	HeLa: 43.00	A549: 32.00
			SW620: 74.00	MCF7: 59.00	FaDu: 25.00
			MDA-MB231: 70.00		
84	*Origanum majorana*	48h	VERO: 70.13	HT29: 13.73	Hep2: 85.63
85	*Hedyosmum spruce*	48h	A549: 44.05	MCF7: 32.76	
86	*Nepeta cataria*	48h	PC3: >500.00	MCF7:> 500.00	DU145: >500.00
87	*Cymbopogon nardus*	24h	HepG-2: 96.60		
88	*Xylopia laevigata*	72h	B16F10: > 25	HepG2: > 25	HL60: > 25
			K562: > 25		
89	*Eugenia egensis*	72h	AGP01: > 25	SKMEL19: > 25	HCT116: > 25
89	*Eugenia flavescens*	72h	AGP01: > 25	SKMEL19: > 25	MRC5: 14.00
			HCT116: 13.90		
89	*Eugenia patrisii*	72h	AGP01: > 25	SKMEL19: > 25	MRC5: 18.10
			HCT116: 16.40		
89	*Eugenia polystachya*	72h	AGP01: > 25	SKMEL19: > 25	MRC5: > 25
			HCT116: 10.30		
90	*Psidium guineense*	48h	MCF7: 44.50	HT29: 29.07	PCO3: 37.55
			NCI-H460: 30.75	K-562: 31.37	OVCAR3: 16.29
			NCI/ADR-RES: 46.09	U251: 45.22	HaCat: 42.82
9	*Garcinia atroviridis*	24h	BEAS2B: 95.00	MCF7: 71	
91	*Pamburus missionis,*	72h	K562: 75.00	DLD1: 365.00	MCF7: 70.00
			A431: 100.00	HL60: 115.00	HepG2: 400.00
			MOLT4: 250.00	HaCaT: 50.00	
92	*Zanthoxylum bungeanum*	48h	HaCaT: 199.20	HeLa: 249.00	HEp2: 174.30
			PC3: 332.00	MFC7: 190.90	
93	*Murraya paniculata*	24h	Hepa1c1c7: 63.70	NIH3T3: 195.00	
94	*Frankincense*	24h	B16F10: 5.00	FM94: 10.00	
95	*Lemongrass*	16h	LU165: 17.35	LU165: 20.93	
			MN1112: 15.28	MN1112: 23.21	

Ref.	Plant name	Exp. Time	Cell lines and related IC_{50} (µg/mL)		
[96]	*Lemongrass*	72h	A549: 1.73	H1975: 4.01	
[68]	*Oliveria decumbens*	24h	MCF7: 0.06	MDA- MB231: 0.14	T47D: 0.10
[97]	*Myrtus communis*	24h	P815: 6.25	MCF7: 4.00	
[98]	*Mesua ferrea*	24h	HCT116: 17.38	LIM1215: 18.86	
[99]	*Satureja hortensis*	24h	A375: 25.00	B164A5: 22.27	
[100]	*Gannanzao*	24h	HepG2: 0.30	HCT116: 0.30	
[101]	*Semenovia suffruticosa*	48h	MCF7:320.00	SH-SY5Y: 160.00	
			HT29: 320.00	NCCIT: 320.00	
[102]	*Mentha spicata*	48h	T47D: 324.00	HCT-116: 279.00	MCF7: 957.00
[103]	*Glandora rosmarinifolia*	72h	HA22T/VGH: 60.50	Hep3B: 61.00	HepG2: 65.00
			MDA-MB231: 46.50	SUM 149: 65.00	
[104]	*Origanum onites*	72h	A375: 8.90	HepG2: 23.00	
			MCF7: 10.00	HT29: 0.35	
[105]	*Curcuma mutabilis Škorničk*	48h	K562: 6.80	HCT116: 8.50	
[69]	*Lavandula stoechas*	24h	MV3: 0.01	MDA-MB231: 0.25	AGS: 0.03
[106]	*Alluaudia procera*	72h	HL60: 25.50	HL60R: 45.80	
[106]	*Meriandra dianthera*	48h	HepG2: 83.60	MCF7: 83.60	
			LoVo: 87.00	HUVEC: 91.20	
[107]	*Nigella Sativa*	48h	HCT116: 43.56	PC3: 29.72	
[108]	*Lawsonia inermis*	72h	HeLa: 0.78	Raji: 0.07	
[109]	*Achillea membranacea*	72h	MCF7: 50.86	HT29: 14.02	A2780: 12.99
[110]	*Zingiber striolatum*	72h	K562: 29.67	PC-3: 86.05	A549: 48.87
[111]	*Zhumeria majdae*	48h	A375: 746.00	MCF7: 674.00	
[106]	*Kalanchoe beharensis*	72h	HL60: 22.00	HL60R: 36.00	
[106]	*Cyphostemma juttae*	72h	HL60: 25.00	HL60R: 36.50	
[112]	*Ferula asafoetida*	48h	HepG2: 7.20	SKHep1: 8.00	
[113]	*Thymus numidicus*	24h	HCT116: 26.90	MCF7: 11.70	
[114]	*Juniperus turbinata*	-h	MDA-MB231: 0.06	HCT116: 0.20	A375: 0.20
[115]	*Stachys viticina Boiss*	24h	HeLa: 1250.00	Colo205: 500.00	
[116]	*Herba Siegesbeckiae*	24h	Hep3B: 37.72	HeLa: 123.16	
[117]	*Sideritis perfoliata*	72h	HeLa: 102.50	UCT-Mel1: 103.15	
			HepG2: 64.27	A431: 133.25	
[118]	*Tea tree*	24h	HEp2: 0.02	A375: 0.03	
[119]	*Trametes suaveolens*	24h	NCI-H460: 24.10	MCF7: 19.20	
[120]	*Citronellol*	48h	A549: 54.02	PC3: 60.83	
[121]	*Aegle marmelos*	24h	PSN-1: 5.60	H157: 6.70	
			LoVo: 6.50	OV2008: 2.30	
[119]	*Bursera glabrifolia*	24h	PC3: 15.20	OVCAR-3: 27.30	K562: 32.40

Ref.	Plant name	Exp. Time	Cell lines and related IC_{50} (µg/mL)		
[75]	*Sideritis raeseri*	72h	A375: 0.15	PC2: 0.21	Caco2: 0.17
[122]	*Melaleuca leucadendra*	72h	MCF7: 70.00	22Rv1: 79.00	EFO21: 98.00
			MCF7/Rap: 71.00	MCF7/4OHTAMO: 55.00	
[123]	*Solidago canadensis*	24h	MDA-MB231: 29.33	HCT116: 18.03	A375: 12.63
[123]	*Solidago gigantea*	24h	MDA-MB231: 18.04	HCT116: 8.10	A375: 5.94
[123]	*Solidago virgaurea*	24h	MDA-MB231: 13.39	HCT116: 8.36	A375: 7.96
[123]	*Solidago ×niederederi*	24h	MDA-MB231: 12.93	HCT116: 6.82	A375: 6.72
[124]	*Citrus × aurantium*	44h	K562: 91.30	MDA-MB231: 74.80	T47D: NA
			MCF7: 82.81	SH-SY5Y: 128.60	ESCs: 184.20
[124]	*Citrus sinensis*	44h	K562: 13.70	MDA-MB231: 39.10	T47D: 43.10
			MCF7: 39.10	SH-SY5Y: 87.90	ESCs: 302.20
[124]	*Citrus limon*	44h	K562: 77.20	MDA-MB231: 37.20	T47D: 19.60
			MCF7: 57.40	SH-SY5Y: 43.90	ESCs: 138.30
[124]	*Boswellia serrata*	44h	K562: 75.40	MDA-MB231: 89.40	T47D: NA
			MCF7: 71.60	SH-SY5Y: 112.90	ESCs: 227.90
[124]	*Boswellia sacra*	44h	K562: 13.70	MDA-MB231: NA	T47D: NA
			MCF7: 231.00	SH-SY5Y: NA	ESCs: 165.40
[124]	*Cistus ladanifer*	44h	K562: 46.90	MDA-MB231: 128.10	T47D: NA
			MCF7: 90.00	SH-SY5Y: 92.80	ESCs: 264.70
[124]	*Aloysia citriodora*	44h	K562: 29.30	MDA-MB231: 56.90	T47D: 113.90
			MCF7: 119.20	SH-SY5Y: 64.50	ESCs: 124.70
[124]	*Foeniculum vulgare*	44h	K562: NA	MDA-MB231: NA	T47D: NA
			MCF7: 165.00	SH-SY5Y: 201.00	ESCs: 152.10
[124]	*Cinnamomum zeylanicum*	44h	K562: 5.20	MDA-MB231: 20.10	T47D: 56.10
			MCF7: 20.80	SH-SY5Y: 21.80	ESCs: NA
[124]	*Syzygium aromaticum*	44h	K562: 89.60	MDA-MB231: NA	T47D: NA
			MCF7: 126.80	SH-SY5Y: NA	ESCs: NA
[124]	*Illicium verum*	44h	K562: 116.10	MDA-MB231: NA	T47D: 171.70
			MCF7: 143.60	SH-SY5Y: NA	ESCs: 213.70
[124]	*Thymus capitatus*	44h	K562: 63.00	MDA-MB231: NA	T47D: NA
			MCF7: 94.10	SH-SY5Y: NA	ESCs: 162.90
[124]	*Cymbopogon citratus*	44h	K562: 57.90	MDA-MB231: 38.40	T47D: 109.50
			MCF7: 98.70	SH-SY5Y: 97.80	ESCs: NA
[124]	*Litsea cubeba*	44h	K562: 11.10	MDA-MB231: 13.40	T47D: 93.70
			MCF7: 32.20	SH-SY5Y: 28.62	ESCs: 96.90
[124]	*Satureja montana*	44h	K562: NA	MDA-MB-231: NA	T47D: NA
			MCF7: 44.00	SH-SY5Y: 98.80	ESCs: 119.30

Ref.	Plant name	Exp. Time	Cell lines and related IC_{50} (µg/mL)		
124	*Thymus vulgaris*	44h	K562: 67.20	MDA-MB231: 61.50	T47D: NA
			MCF7: 39.90	SH-SY5Y: 49.30	ESCs: 152.70
125	*Croton matourensis Aubl*	72h	MCF7: 23.30	HL60: 17.80	

Anticancer activity of 19 EOs against the digestive system associate cancer cell lines is given in Table 4. The most potent EO with IC_{50} (72h) 0.03 µg/mL against HT29 is *Ocimum viride* [126]. The second potent EO with IC_{50} of 1.54 µg/mL against ACP03 is *Piper aequale* [127]. Other EOs with acceptable IC_{50} against MIA PaCa-2: 11.00 µg/mL and HSC3: 13.70 µg/mL are *Aquilaria crassna* and *Cinnamomum cassia*, respectively [128, 129]. Similar to the previous section, the IC_{50} of other EOs is substantially higher than commercial drugs.

Table 5: Researches that were targeting digestive system associate cell lines

Ref.	Plant name	Exp. Time	Cell lines and related IC_{50} (µg/mL)	
130	*Heracleum mantegazzianum*	48h	VERO: 302.80	SCC25: 567.80
			HEK293: 262.30	FaDu: 380.20
127	*Piper aequale*	72h	HCT116: 8.69	ACP03: 1.54
131	*Illicium verum*	48h	HCT116: 50.34	HT29: 100.00
132	*Myrica rubra*	72h	CaCo2: 51.00	PDL: 55.00
133	*Origanum vulgare*	24h	HepG2: 236.00	HEK293: 310.00
134	*Lavandula hybrid Rev*	-*	Caco2: 913.00	
134	*Lavandula latifolia*	-*	Caco2: 779.00	
134	*Lavandula vera D.C.*	-*	Caco2: 1224.00	
134	*Lavandula angustifolia*	-*	Caco2: 1631.00	
129	*Cinnamomum cassia*	48h	HSC3: 13.70	
135	*Origanum dictamnus*	24h	LoVo: 84.76	
136	*Thymus caramanicus*	24h	KB: 440.00	
128	*Aquilaria crassna*	48h	MIA PaCa2: 11.00	
126	*Ocimum viride*	72h	HT29: 0.03	
137	*Origanum vulgare*	48h	AGS: 13.40	
138	*Origanum majorana*	48h	HT29: 142.00	
139	*Mentha citrata*	48h	HCT116: 80.60	
140	*Cannabis sativa*	24h	HCT116: 500	
141	*Cotula cinerea*	-*	Vero: 72.72	

*Exposure time has not been reported.

Table 5 demonstrates the anticancer activity of 19 EOs against genital organs associated with cell lines. Among the EOs, all *Piper* species show excellent activity against HeLa (~0.02 µg/mL) with an exposure time of 24h [142]. This value is comparable to drugs in Table 2. For instance, IC_{50} of doxorubicin with similar exposure time (i.e., 24h) is 4.50 µg/mL [30].

Table 6: Researches that targeted cell lines associated with genital organs

Ref.	Plant name	Exp. Time	Cell lines and related IC_{50} (µg/mL)	
[143]	*Dracocephalum kotschyi*	48h	HeLa: 26.40	
[142]	*Piper betle*	24h	HeLa: 0.02	
[142]	*Piper betloides*	24h	HeLa: 0.03	
[142]	*Piper crocatum*	24h	HeLa: 0.02	
[142]	*Piper maculaphyllum*	24h	HeLa: 0.03	
[142]	*Piper rubrograndulosum*	24h	HeLa: 0.03	
[142]	*Piper semiimmersum*	24h	HeLa: 0.03	
[142]	*Piper submultinerve*	24h	HeLa: 0.02	
[142]	*Piper tricolor*	24h	HeLa: 0.02	
[142]	*Piper yinkiangense*	24h	HeLa: 0.02	
[144]	*Thymelaea hirsute*	-*	HeLa: 175.00	
[145]	*Atalantia monophylla*	48h	HeLa: 43.08	
[146]	*Artemisia ciniformis*	48h	HeLa: 19.64	
[147]	*Rosmarinus officinalis*	24h	HeLa: 909.60	
[147]	*Curcuma longa*	24h	HeLa: 211.60	
[147]	*Zingiber officinale R*	24h	HeLa: 141.40	
[148]	*Lavender angustifolia*	48h	PC3: 1990.00	DU145: 370.00
[149]	*Cymbopogon nardus*	24h	LNCaP: 58.00	
[150]	*Chenopodium Botrys*	24h	HeLa: 75.00	

*Exposure time has not been reported.

From Table 6, information about the anticancer activity of 19 EOs against breast, lung, and skin cancer cell lines is available. Potencies (IC_{50}) of *Inula japonica* and *Angelicae dahuricae* EOs against MCF7 are 0.36 and 0.40 µg/mL, respectively, and against MCF7/ADR are 3.68 and 5.37 µg/mL respectively [151]. IC_{50} of paclitaxel and doxorubicin with the same exposure time of 48h against MCF7 are 3.45 and 2.10 µg/mL, respectively [29, 38].

Table 7: Researches that targeted breast, lung, and skin cancer cell lines

Ref.	Plant name	Exp. Time	Cell lines and related IC_{50} (µg/mL)	
[152]	*Citrus reticulate*	24h	A549: 96.00	
[153]	*Artemisia arborescens*	72h	A375: 6.70	
[154]	*Artemisia gmelinii*	24h	A549: 125	
[155]	*Croton tiglium*	24h	A549: 48.38	
[156]	*Thymus bovei Benth*	72h	A549C5: 8.62	
[157]	*Blepharocalyx salicifolius*	48h	MDA-MB231: 46.60 MCF10A: 314.44	MCF7: >512
[158]	*Zataria multiflora*	48h	MDA-MB231: 29.89 MCF7: 25.06	T47D: 20.09

Ref.	Plant name	Exp. Time	Cell lines and related IC_{50} (µg/mL)	
[159]	*Teucrium yemense*	72h	MDA-MB231: 59.90	MCF7: 24.40
[160]	*Nepeta menthoides*	-*	MDA-MB231: 1243.00	T47D: 1934.00
				MCF7: 424.00
[151]	*Inula japonica*	48h	MCF7/ADR: 3.68	MCF7: 0.36
[151]	*Angelicae dahuricae*	48h	MCF7/ADR: 5.37	MCF7: 0.40
[161]	*Decatropis bicolor*	24h	MDA-MB231: 53.81	MCF10A: 207.51
[162]	*Ocimum sanctum*	24h	MCF7: 170.00	
[163]	*Pinus densiflora*	48h	MCF7: 90.20	
[164]	*Rosmarinus officinalis*	72h	MCF7: 200	
	Thymus vulgaris L.	72h	MCF7: 100	
	Lavender x intermedia	72h	MCF7: 300	
[165]	*Cordia africana*	24h	MCF7: 12.90	
[166]	*Garcinia celebica*	48h	MCF7: 45.20	

*Exposure time has not been reported.

4. Future perspectives

Until recently, plants have been a source of active pharmaceutical compounds and blockbuster drugs. Despite synthetic drugs, medicinal plants' share in treating and preventing various diseases such as diabetes, hypertension, and cancer is still enormous [167]. Drug discovery, based on the biological activity from medicinal plants, led to the isolation of anticancer drugs such as taxol (generic name of paclitaxel), camptothecin, etc. [168]. Taxol was isolated from the bark of *Taxus brevifolia*, also known as Pacific yew tree, through a program initiated by the National Cancer Institute (NCI) in 1958 to screen plants for anticancer activity [169, 170]. After determining its structure and passing clinical trial phases I and II, its commercialized product was introduced in 1991 [171, 172]. Nowadays, taxol has been identified as a vital chemotherapy drug. It is approved by the Food and Drug Administration (FDA) to treat different cancers such as ovarian, breast, and lung cancers [171]. Vinblastine and vincristine are two other well-known chemotropic drugs isolated from the *Catharanthus roseus* in the 1960s [173]. Vinblastine and vincristine are used to treat Hodgkin's disease (a form of lymphoid cancer) and children's leukemia, respectively [174].

Safety, efficacy, and structural diversity are the most prominent features of natural products compared with compounds derived from computational and combinatorial chemistry [168]. On the other hand, severe side effects and reduced quality of life in common anticancer treatments such as surgery, radiation therapy, and chemotherapy leaves no doubt that we need to discover new efficient anticancer drugs, and plants are the first and most suitable choice [175]. We believe that other plants with comparable anticancer activities with commercial agents need to be explored more. For instance, in Tables 3-7, some EOs with excellent anticancer activities (IC_{50}: < 1 µg/mL) against at least a cancer cell are observable. They include *Pinus eldarica* [65], *Pallenis spinosa* [66], *Pallines spinosa* [67], *Foeniculum vulgare* [76], *Oliveria decumbens* [68], *Gannanzao* [100], *Origanum onites* [104], *Lavandula stoechas* [69], *Lawsonia inermis* [108], *Juniperus turbinate* [114], *Sideritis raeseri* [75], *Ocimum viride* [126], *Piper* spp. (including betle, *P. betloides, P. crocatum, P. maculaphyllum, P. rubrograndulosum, P. semiimmersum, P. submultinerve, P. tricolor,* and *P. yinkiangense*) [142], *Inula japonica* [151], and *Angelicae dahuricae* [151]. Mentioned EOs appear to be good

candidates, and their anticancer activity should be evaluated against commercial drugs.

Furthermore, using herbal food additives such as cinnamon, ginger, and turmeric is common worldwide; interestingly, their anticancer activity is acceptable [176-178]. Additionally, using other herbal products such as curcumin and eugenol as (supplementary) drug(s) is usual [179, 180]. Therefore, although most plants' effectiveness is not comparable to commercial medications, they can be considered supplementary medicine or suggested to be used as food additives.

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Conflict of Interest

There is no conflict of interest with the authors.

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التقييم في المختبر للزيوت الأساسية كعوامل علاجية مضادة للسرطان: مراجعة منهجية للأدبيات

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ملخص

السرطان مرض قاتل يسبب حوالي 9 ملايين حالة وفاة سنويا في البلدان النامية والمتقدمة في جميع أنحاء العالم. أصبحت المنتجات الطبيعية مؤخرًا كبدائل للعوامل الكيميائية مجال اهتمام متزايد. الزيوت الأساسية هي مستقلبات ثانوية للنبات، مع مجموعة واسعة من الأنشطة الحيوية، مثل التأثير المضاد للسرطان. تحاول المراجعة المنهجية الحالية جمع وتوثيق الدراسات الحديثة من 01.01.2016 إلى 12.31.2020، والتي تشير إلى الزيوت الأساسية كعوامل مضادة للسرطان في الدراسات المختبرية؛ تم استخراج بيانات 144 تقريرًا. تم تلخيص التأثيرات المضادة للسرطان لـ 187 زيتًا أساسيًا متميزًا على 112 خطًا خلويًا. هذا بنك قيم للباحثين الذين يجدون الزيت العطري المناسب كعامل مضاد للسرطان. تم اقتراح بعض الزيوت الأساسية التي لها تأثيرات مماثلة مع الأدوية التقليدية. هذه الزيوت الأساسية هي مرشحة جيدة لمزيد من الدراسات، مثل التحقيقات في الجسم الحي.

الكلمات الدالة: مراجعة منهجية، زيت عطري، نشاط مضاد للسرطان، دراسات مخبرية، مواد مضافة للغذاء.

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A Comparative Cross-Sectional Study- Knowledge, behavior and psychological change among Medical and Non-medical Students in Jordan during COVID-19 pandemic

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) has rapidly spread worldwide, and it was officially declared to be a pandemic by the World Health Organization on March 11, 2020. COVID-19 is associated with increasing morbidity and mortality and has impacted the lives of the global populations.

Aim: To compare the knowledge of medical and non-medical students at Jordanian universities in issues related to COVID-19 and to evaluate the psychological and behavioural changes in Jordanian students' lives following directly/indirectly exposure to the COVID-19.

Methods: A descriptive cross-sectional online survey was sent to a convenience sample in Jordanian universities through social media (Facebook and WhatsApp) between 16th of June and 30th of June 2020. The questionnaire was designed to collect the demographic, participant's source of information regarding COVID-19, knowledge on COVID-19, the psychological consequences of COVID-19, impact of COVID-19 on participant's behaviour. The final version of the questionnaire was further tested for content validity by experts in the field. Chi-square test was used to find significant differences between the two groups.

Results: A total of 912 participants completed the survey, with 507(55.6%) being medical students and 405(44.4%) being non-medical students. About 90% of students believed in the existence of corona virus (n=817), but not in the seriousness of the infection (n=85, 9.3%). The majority 82.2% (n=750) agreed that the fake news on social media caused panic situations. A total of 275 medical students avoided following news as compared to 187 non-medical students, and the difference was statistically significant (p-value = 0.003). There were 438 medical students and 338 non medical students who avoided leaving the house for unnecessary needs (p-value = 0.004).

Conclusion: Medical students had better knowledge and were more aware on COVID-19 than that of non-medical students; for this reason, medical students tended to change their behaviours in a good way. The current pandemic seems to impact the psychology of the both groups with no difference significant.

Keywords: Covid-19, medical students, Knowledge, psychological impact, behaviour.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an emerging respiratory disease caused by a novel coronavirus [1]. COVID-19 was first detected in December 2019 in Wuhan, China [2]. On January 30, 2020, the World Health Organization (WHO) declared that COVID-19 is a pandemic disease [3]. As of December 2019, until May 31, 2021, about 170 million cases were reported globally and more than 3.5 million deaths [4].

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COVID-19 can spread to humans through intermediate host such as bats or from human-to-human, through respiratory droplets and body contacts [1]. Contact with contaminated surfaces, hands, and touching of faces, eye, nose, mouth are predominant ways to get exposed to the infected droplets [5]. The main signs and symptoms of COVID-19 include fever, cough, and shortness of breath [6]. While the incubation period of COVID-19 is between 2–14 days after exposure [4]. Preliminary data suggest that older adults and persons with underlying health conditions or compromised immune systems might be at greater risk for severe illness from this virus [7].

Jordan recorded the first case of COVID-19 in March 2020[8]. Jordan witnessed a sudden increase in the number of infected people during the past threemonths (March, April and May) with a peak of more than 9,000 cases on the 22th March 2021[4].

In order to suppress this pandemic, the government enforced strict quarantine measures and initiated a public information campaign utilizing various media to alert citizens about the dangers of the virus. Many studies have shown that practicing preventive measures on an individual level is effective in curbing the spread of infection [3], i.e. wearing a mask, washing hands, using a hand sanitizer, maintaining social distancing, and staying at home [3].On the other hand, the world health organization (WHO) released some mental health considerations that should be followed during this crisis such as; avoid watching and listening to news constantly, staying connected with loved ones through digital media, reassuring and supporting each other, along with taking care of one's own health i.e. exercising, eating healthy, and sleeping well regularly [9].This in turn, can affect the behavioral and psychological response of the population[10-11].

As Jordan is a youthful country, university students account for the biggest proportion of the people. Hence, we must pay attention to study their knowledge about COVID-19 especially after the large amount of misconceptions and false information that are circulating on social media regarding transmission of the disease and methods of acquisition[12]. As well as the impact of this pandemic on their behavior and psychological responses[13].

As our study is novel, the findings of this study may inform health officials on further interventions, awareness, and policy improvements pertaining to the COVID-19 outbreak in Jordan, at this critical time.

Aim of the study

This study aimed to compare the knowledge of medical and non-medical students at Jordanian universities in issues related to COVID-19. Additionally, this study aimed to evaluate the psychological and behavioral changes in Jordanian students' lives following directly/indirectly exposure to the COVID-19.

Ethical approval

This study was approved by the Research Ethics Committee at Faculty of Pharmacy in ASU, Amman, Jordan (No: 2020-PHA-25). The consent to participate was implied by the act of completing and returning the e-survey.

Methods

Study design and population

A descriptive cross-sectional online survey was conducted on the16th of June 2020 in Jordan.

Questionnaire development and data collection

Following an extensive literature review on studies related to COVID-19 pandemic, a draft questionnaire was designed to cover the areas of our interest in this study [5-8-13-14]. The questionnaire was written in two languages; it was written in the English language because it is the medium of instruction in Jordanian universities and was translated to the Arabic language as Arabic is the first language in Jordan. The translation was validated by the Translation Department at the Applied Science Private University (ASU) followed the standard 'forward-backward' procedure. The final version of the questionnaire was further tested for content validity by experts in the filed who gave their constructive suggestions, positive feedback for the process. The final version of the questionnaire was organized into five main sections addressing different topics of interest. Section I

included items to collect demographics data about students' characteristics. The students in the second section were asked a multiple-choice question about their source of information about COVID-19. Section III was planned to assess participants knowledge and believes about COVID-19 pandemic. Section IV was to assess the psychological consequences of COVID-19 pandemic on study participants. Finally, section V was to assess the behavioral impact of COVID-19 on the Jordanian students.

Sampling strategy and sample size

A convenience sample of eligible participants was invited to participate in the study from governmental and private Jordanian universities through social media (Facebook and WhatsApp). The covering letter stressed anonymity and confidentiality and explained aim and objectives of this study. The participants did not receive any benefit or payment for filling-out the questionnaire.

Based on a total university student population of 377,000[14], sample size calculation using a margin of error of 5%, confidence level of 95%, and response distribution of 50%, a minimum sample size of 384 of people is needed.

Statistical analysis

Data were analyzed by using statistical package for social science (SPSS) version 26. The descriptive analysis was performed using frequency/percentage for qualitative variables. Age variable was expressed as mean ± SD. The chi-square test was used to describe the statistical differences between medical and non-medical students groups. P-values <0.05 indicated that a difference was statistically significant.

Results

Participants' characteristics

A total of 912 students completed the survey. Students' characteristics are shown in **Table 1**. The mean age of the participants was 23.83 ± 1.50 years, ranging from 18 to 26 years. The majority of students were male (n=656, 71.9%). A large fraction of our participants was from urban areas (n=866, 94.9%). There were 851 (93.3%) of students were undergraduate while the number of post-graduate students was 61(6.7%). About half of students were studying in health/medical field (n=507, 55.6%) and 405 students (44.4%) were studying in other fields. Approximately, all students were committed in home quarantine (n=844, 92.5%).

Table 1: Demographic characteristics of the study sample (n=912)

Age (M ± SD)	23.83 ± 1.50
Academic level n(%)	
post-graduates	61(6.7)
Undergraduates	851(93.3)
Gender n(%)	
Male	656(71.9)
Female	256(28.1)
Accommodation place n(%)	
City	866(94.9)
Urban	46(5.1)
Field of study n(%)	
Medical field	507(55.6)
Non-medical field	405(44.4)
Students infected previously by n(%) corona	12(1.3)
Students committed in home quarantine	844(92.5)

Participants' sources of information about COVID-19

Students' sources of information about the COVID-19 pandemic **(Table 2**) were varied between social media platforms which presented the most common sources of information (38.4%), followed by visual and audible media (34.1%). Unexpectedly, academic research presented only 13.2% of information resources, while, the world health organization (WHO) reports were reported to be the least common source of information (1.0%).

Table 2: Sources of information about COVID-19 among the study participants (n=912)

Source	Percent
Academic research	13.2
Social media	38.4
Visual and audible media	34.1
Public's conversation	4.6
WHO reports	1.0

Participants knowledge and believes about COVID-19 pandemic

Interesting results were revealed and outlined in **Table 3** about knowledge of the two groups on COVID-19 pandemic. About ninety percent of students believed in the existence of corona virus (n=817), but not in the seriousness of the infection (n=85, 9.3%). The majority of the students knew well the common symptoms of COVID-19 infection in the respiratory system (n=842, 92.3%). However, the students were evenly split between those who knew the symptoms of the infection on the digestive system (n=436, 47.8%) and who did not. Unfortunately, students expressed less knowledge about the effect of antihypertensive medications (20.4%) antibiotics (31.0%) and Hydroxychloroquine (36.6%) on the COVID-19 infection.

Table 3: Students' knowledge and beliefs about COVID-19 pandemic (n=912)

Statements	Total correct answer N(%)	Medical students N	Non-medical students N	χ^2	p-value*
Corona virus is a biological weapon.	567(62.2)	290	277	9.558	0.002*
Corona virus is a real fact.	817(89.6)	470	347	14.990	<0.001*
Corona virus infection is a serious disease.	85(9.3)	43	42	13.728	0.001*
Common symptoms of corona include shortness of breath, coughing, and sore throat, accompanied by a very high fever.	842(92.3)	474	368	10.148	0.006*
The drugs hydroxychloroquine is effective in treating corona infection.	334(36.6)	188	146	30.814	<0.001*
Antibiotics play an effective role in the recovery of COVID-19.	283(31.0)	169	114	33.163	<0.01*

Statements	Total correct answer N(%)	Medical students N	Non-medical students N	χ^2	p-value*
The risk of antihypertensive in infection with COVID-19	186(20.39)	100	86	4.666	0.202
Heat and hot weather can eliminate the Corona virus.	501(54.9)	305	196	25.794	<0.001*
Cold weather and snow cannot kill the Corona virus.	437(47.9)	231	206	15.194	<0.001*
Corona virus does not affect children under the age of 16	785(86.1)	449	336	21.808	<0.001*
The incubation period of Corona virus is less than a week.	239(26.2)	146	93	15.648	<0.001*
An infected person with Corona virus can develop diarrhea and intestinal pain.	436(47.8)	275	161	30.492	<0.001*
A person can develop corona without showing symptoms.	776(85.0)	450	326	32.761	<0.001*
Corona infection might leads to death.	137(15.3)	80	57	14.476	<0.001*
The disease is transmitted from one person to another by touching places contaminated with the infected person's secretions by the hand, then touching the eyes, mouth, and nose.	838(91.8)	481	357	12.969	0.002*
Corona virus is believed to be spread mainly from person to person through respiratory droplets that result when the infected person coughs or sneezes	269(29.5)	165	104	8.628	0.013*
The person infected with Corona virus can transmit the disease during the incubation period.	773(84.8)	453	320	44.055	<0.001*
Most of the deaths caused by the Corona virus are for the elderly and those suffering from chronic diseases.	834(91.4)	475	359	22.295	<0.001*
People who have contacted with the patient infected with Corona virus should be banned for 14 days.	841(92.2)	474	367	4.604	0.100
Many pre-existing medications are tried to relieve symptoms, rather than treat them.	686(75.2)	281	405	26.758	<0.001*
Focusing on strengthening the immune system can prevent the infection with Corona virus.	712(78.1)	417	295	6.164	0.46
Entering the hospital can accelerate the recovery of patient with Corona virus.	580(63.6)	326	254	1.661	0.436
A single mask can be used multiple times.	736(80.7)	422	314	2.278	0.320
All family members can use the same mask.	837(91.8)	475	362	8.958	0.011*

*Chi square as a test of significance, p-value<0.05

In this section the majority of questions were significantly different between the two groups. Medical students were more knowledgeable than non-medical students regarding the questions: seriousness of the disease, symptoms of the COVID-19 infection, effectiveness of drugs, influence of weather on the corona virus, and transmission the disease (p-value < 0.05). Non-medical student (n=405, 44.4%) tended to answer correctly for only one question " Many pre-existing medications are tried to relieve symptoms, rather than treat them" more than medical students (n=185, 20.3%) and the difference was statistically significant (p < 0.001).

The psychological effect of COVID-19 pandemic on study participants.

Table 4. depicted the psychological effects of the ongoing pandemic among Jordanian students. The vast majority (84.6%, n=772) of students were afraid of visiting crowded places and 58% (n=529) reported fear of leaving their homes. Almost all students (93.7%, n=855) expressed concerns about safety of their families. The majority 82.2% (n=750) agreed that the fake news on social media caused panic situations and 67.3% (n=614) reported that they believe the situation is not as bad as portrayed by the media.

Table 4: The proportion of students who expressed agreement about the psychological effects of COVID-19 (n=912)

Statements	Total agreement n(%)	Medical students	Non-medical students	χ^2	p-value*
I am afraid to leave my home because of the Corona virus.	529(58.0)	295	234	0.470	0.493
I am afraid to visit crowded places, i.e. stores and markets	772(84.6)	433	339	1.027	0.311
I fear for my health even when I am at home.	368(40.3)	204	164	1.041	0.308
I fear for the health of my family.	855(93.7)	479	376	3.171	0.075
I am concerned when a family member walks out.	732(80.2)	412	320	0.891	0.345
I feel that the government should isolate patients infected with the Corona virus in specific hospitals.	842(92.3)	473	369	2.208	0.137
I feel unsure about the current infection control measures.	392(42.9)	221	171	1.170	0.279
I feel the fake news appearing on social media regarding Corona virus causing panic.	750(82.2)	421	329	0.404	0.525
I feel the situation is not as bad as being portrayed by the media.	614(67.3)	320	294	6.024	0.140

*Chi square as a test of significance, p-value<0.05

There was no statistically significant difference in the questions between the two groups in this section.

The behavioral impact of COVID-19 on study participants.

This section aimed to evaluate the impact of COVID-19 pandemic on students' behavior in terms of abiding with the government recommendations about wearing masks, keeping social distancing and personal hygienic habits. The results are demonstrated in **Table 5.**The vast majority of students avoided the use of public facilities such as prayer places (88.9%),

gardens and buses (90.3%). Most of students changed their social habits to new ones, such as hand shakinghabits (85.9%), limiting the physical contact (85.3%) with others and family reunions (84.2%). Similarly, most of students changed their personal habits such as wearing masks (89.4%), washing hands repeatedly (83.4%), quitting smoking (55.8%) and carrying a hand sanitizer (80.7%) due to the COVID-19 pandemic.

Table 5: The proportion of students who changed their behavior because of COVID-19 (n=912)

Statements	Total Agreement N(%)	Medical students	Non-medical students	χ^2	p-value*
I thought about quitting smoking (or any other bad habits).	509(55.8)	315	194	11.286	<0.001*
I have thought applying for leave in the course due to COVID-19.	358(39.2)	215	143	3.904	0.048*
I claimed to be sick to avoid going to the course	132(14.4)	76	56	0.546	0.460
I have identified physical contacts with people.	778(85.3)	438	340	1.567	0.211
I recently avoided using public health care facilities.	824(90.3)	460	364	1.209	0.271
I recently avoided going to prayer places.	811(88.9)	458	353	5.359	0.021*
I have recently started avoiding watching, reading, or listening to news because it makes me anxious.	462(50.6)	275	187	8.781	0.003*
My planes were recently canceled, i.e. family reunions, social gathering, travel or meeting due to COVID-19	768(84.2)	429	339	1.173	0.279
I have recently purchased a lot of groceries fearing of running out of food	426(46.7)	261	165	2.830	0.092
I wash my hands more frequently than before.	761(83.4)	439	322	4.392	0.036*
I carry hand sanitizer all the time	736(80.7)	437	299	34.189	<0.001*
I started to wear a mask because of corona virus.	816(89.4)	465	351	4.092	0.043*
I used to by my basic needs using delivery service.	478(52.4)	298	180	16.370	<0.001*
I avoid shaking hands or hugging, and just greeting the head	784(85.9)	446	338	6.953	0.008*
I always wear a mask everywhere outside the home.	729(79.9)	420	309	2.670	0.102
I avoid leaving the house for unnecessary needs .	776(85.0)	438	338	8.391	0.004*

*Chi square as a test of significance, p-value<0.05

Among medical students, there was an overall agreement in responses 1.5-2 fold more than non medical students. A total of 275 medical students avoided following news as compared to 187 non-medical students, and the difference was statistically significant (p-value = 0.003). There were 438 medical students and 338 non medical students who avoided leaving the house for unnecessary needs (p-value = 0.004).

Discussion

Since the outbreak of COVID-19 almost two years ago, it has been documented that the pandemic effects are not restricted to physiological deterioration and death of infected patients. The whole life style and mental health of the public have been impacted, especially students who have lived with greater concerns of losing their future given the new learning methods in universities. Therefore, this study aimed to investigate what medical and non-medical students know about COVID-19 and how this pandemic affected their behaviours and mental health.

Overall, only few students were relying on academic research as a source of information about COVID-19 and most of the students indicated that they get COVID-19 information from social media and other media websites. This outcome could be explained by two ways. First, academic

research entities such as journals and publishers are not significantly involved in social media websites. Second, in Jordan, students in general do not receive adequate training on how to get information from a trusted source. Our study aligns with a previous study in Jordan, which indicated that social media represents the most common source of information about COVID-19 among university students [14]. Accordingly, students need to be trained to how to filter information, especially those with medical background and journals need to actively implement programmes that target students on social media.

As expected, medical students had better knowledge on COVID-19 and this could be explained by the higher exposure to medical information as part of their studies. Nonetheless, both medical and non-medical students had some gabs in their knowledge. Surprisingly, previous studies showed that both medical and non-medical students in Jordan had very good knowledge on COVID-19 [14,15]. The studies in the literature showed variation in the levels of knowledge on COVID-19 among university students [16,17]. There are several factors that could contribute to these findings. First, students in different countries are exposed to different COVID-19 information based on local governments. Second, some of the information about COVID-19 are still controversial and the students' confusion toward this information is justified given the conflicting reports about it.

Our findings indicated that most of the students have good knowledge on COVID-19. For example, our students had good knowledge on COVID-19 symptoms, but not the impact of COVID-19 on the digestive system. The students were afraid about the safety of their families and they were afraid of visiting crowded places or even leaving homes. Additionally, our findings showed an overall change in behaviour of students. This includes social and cleaning habits. These outcomes could be attributed to the global economic and social consequences of COVID-19. Previous studies linked fears and uncertainty with diseases like depression, anxiety, and the feeling of stigmatization[18,19,20,21].

In summary, this study provides health officials in Jordan with a comprehensive assessment of students' knowledge on COVID-19. Additionally, it emphasizes how COVID-19 changes the behaviour of students. Nevertheless, further studies with a larger sample size are necessary to shed more light on this topic.

This study has several limitations. First, the sample frame was not representative and we believe that there are many students with different level of knowledge might be missed in our study. Second, self-reporting could induce bias.

Conclusion

Medical students had better knowledge and were more aware on COVID-19 than that of non-medical students; for this reason, medical students tended to change their behaviors in a good way. The current pandemic seems to impact the psychology of the both group with no difference significant.

Conflicts of interest

The authors declare that they have no conflicts of interest to disclose.

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المعرفة والسلوك والتغيير النفسي بين طلاب الكليات الطبية وغير الطبية في الأردن خلال جائحة COVID-19

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ملخص

الخلفية: انتشر مرض فيروس كورونا 2019 (COVID-19) بسرعة في جميع أنحاء العالم، وأعلنت منظمة الصحة العالمية رسميا أنه وباء في 11 مارس 2020. ويرتبط COVID-19 بزيادة معدلات الاعتلال والوفيات، وقد أثر على حياة سكان العالم. الهدف: مقارنة معرفة طلاب الطب وغير الطبيين في الجامعات الأردنية في القضايا المتعلقة ب COVID-19 وتقييم التغيرات النفسية والسلوكية في حياة الطلاب الأردنيين بعد التعرض المباشر/غير المباشر ل .COVID-19

الطرق: تم إرسال استطلاع وصفي عبر الإنترنت إلى عينة ملائمة في الجامعات الأردنية من خلال وسائل التواصل الاجتماعي (فيسبوك وواتساب) بين 16 يونيو و30 يونيو 2020. وقد صمم الاستبيان لجمع المعلومات الديمغرافية، ومصدر معلومات المشاركين فيما يتعلق بCOVID-19 ، والمعرفة بشأنCOVID-19 ، والعواقب النفسية لCOVID-19 ، وتأثير COVID-19على سلوك المشاركين. واختبر الخبراء في الميدان الصيغة النهائية للاستبيان من أجل صحة المحتوى. تم استخدام اختبار Chi-square للعثور على اختلافات كبيرة بين المجموعتين .

النتائج: أكمل ما مجموعه 912 مشاركا الدراسة الاستقصائية، وكان 507 (55.6 في المائة) من طلاب الطب و405 (44.4 في المائة) من غير طلاب الطب. حوالي 90٪ من الطلاب يؤمنون بوجود فيروس كورونا(n = 817) ، ولكن ليس في خطورة العدوى (ن = 85، 9.3٪). ووافقت الأغلبية 82.2٪ (ن= 750) على أن الأخبار المزيفة على وسائل التواصل الاجتماعي تسببت في حالات ذعر. تجنب ما مجموعه 275 طالب طب متابعة الأخبار مقارنة ب 187 طالبا من غير طلاب الطب، وكان الفرق مهما إحصائيا) القيمة .(p = 0.003 كان هناك 438 طالب طب و338 طالب طب تجنبوا مغادرة المنزل لتلبية احتياجات غير ضرورية) قيمة.(p = 0.004

استنتاج: كان لدى طلاب الطب معرفة أفضل وكانوا أكثر وعيا ب COVID-19 من الطلاب غير الطبيين؛ لهذا السبب، يميل طلاب الطب إلى تغيير سلوكهم بطريقة جيدة. ويبدو أن الوباء الحالي يؤثر على نفسية المجموعتين دون فرق كبير.

الكلمات الدالة: Covid-19، طلاب الطب، والمعرفة، والأثر النفسي، والسلوك.

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Emergency Research Status in the Middle Eastern Region

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ABSTRACT

Conducting research in emergency settings is paramount to advance knowledge and experiences on dealing with critically ill patients. However, the involvement of highly vulnerable subjects, who are mostly incapable of consenting to research participation is challenging. This research paper describes the extent of emergency research and its related ethical issues within emergency settings in the Middle East. A literature review covering several databases was conducted. Several studies conducted within the emergency departments in the Middle Eastern region were located. All studies focused on provision of emergency care, while none described aspects of emergency research. Current literature indicates a dearth of research concerning emergency research settings in developing countries mainly in the Middle Eastern region. Up to the authors' knowledge, this is the first research paper to review the available literature on emergency research in the Middle East. Findings from this review suggests that more efforts focusing on emergency research should be encouraged and stimulated in the region, in order to improve the effectiveness and safety of healthcare provision to critically ill patients within emergency departments. Adding to that, our findings stimulate the needs for regulatory bodies and regulations that help overcome the main ethical issues surrounding the principle of obtaining informed consents in emergency settings.
Keywords: Emergency; Research; Middel East; Ethics.

INTRODUCTION

Provision of healthcare in emergency settings should be based on best research evidence to ensure safe, and effective procedures/treatments[1]. Therefore, conducting research in emergency settings is important to advance knowledge and experiences on dealing and/or treating critically ill patients[2]. Corresponding to that, a report in 2016 showed that the National Institutes of Health support more studies covering clinical emergency care research[3]. Emergency settings involve highly vulnerable subjects, who are mostly incapable to provide consent to medical procedures and / or research participation[4]. Such emergent problems mandate immediate actions, where healthcare providers are responsible for assuring the provision of effective and safe interventions[5]. Given the complexity of situations, it would be highly demanding to obtain informed consents (in a timely manner) from unstable patients or their legal representatives in order to provide them with required interventions such as Cardio-Pulmonary-Resuscitation (CPR)[6]. As a result, such situations represent a substantial ethical challenge to healthcare providers and researchers who basically deal with emergency cases[7].

Worldwide, since the mid of 70's and according to the Belmont report, researchers and healthcare practitioners must respect the utmost importance of subject's autonomy "respect for the patient's capacity of self-determination, and exercise of personal choice, " [8]represented by the

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principle of informed consent[9]. However, the problematic situation with emergency research originates from the fact that it is not always possible to obtain informed consents from patients who are unconscious, unable to communicate with healthcare providers or researchers, or undergoing tremendous physical and/or psychological stress [2, 10]. Because outcomes in emergency settings depend mainly on the earliest provided intervention, any delay (even short) to obtain consent (including proxy consent) may cause mortality or loss of survival chances[11]. In accordance to that the Federal Drug Administration [12] established a policy in 1996 "Exception from Informed Consent (EFIC)" as an attempt to balance between human right of "Autonomy" and the progress of medical practices and research in emergency settings[13]. In addition to that the FDA still mandates several protective measures to emphasize on patients autonomy[14]. First of all, FDA articulates conditions for applying EFIC policy. Such conditions include unexpected case scenarios with life threatening conditions, such as dealing with debilitated or incapacitated patients, lack of proven or satisfactory treatment options, availability of interventions that might achieve direct benefit to the health of patients, feasibility of offering treatments on timely manner (prior to obtaining proxy consent from legally authorized representatives), and community consultation. Such requirements, however, remains unclear and poorly defined to both emergency healthcare practitioners and researchers. Studies have revealed high levels of frustration among emergency researchers within some settings, where physicians have a full power to prescribe, even without an ethical approval, pharmaceuticals that have not been scientifically approved effective and safe for certain medical conditions [7, 15, 16].

Besides researchers' and healthcare providers' opinion about waiving informed consent in emergency settings, many studies have shown that public's opinion and acceptance to this principle is vague. There is no clear answer to the question of as to whether available regulations that allow for waiving informed consent in emergency settings could protect public health and human rights [17]. Worldwide, it is highly recommended to educate populations about medical research within different settings and the importance of public involvement in research. Shedding light on factors that can influence and / or hinder public trust in medical research especially in research without consent is highly needed.

Even though emergency departments in developing countries serve very large populations with increased levels of mortality and critical illnesses[18, 19], little or no regulations and rules that demarcate emergency practices and research principles are available. As an example, the Jordanian Clinical Research Law of 2001, covers none regarding emergency research and its ethical related issues [20, 21]. This research paper aimed to review research examining emergency research and challenging ethical issues in emergency situations within developing countries, focusing on Middle East. Application of different ethical rules and regulations permitting research of this kind within this region was the main focus of the current search.

Research Methods

Several databases were searched (September 2020 - February 2021): PubMed, Web of Science, Medline, PsychInfo, Web of Knowledge, and CINAHL. The search terms are listed in Table 1. Research papers were located and individually reviewed by both researchers. Suitability of research papers to be included was based on detailed discussions and agreement between researchers. Final agreement was to locate all peer reviewed papers covering any topic related to emergency medicine and/or research within the Middle Eastern region. References lists of located papers were searched to identify relevant papers. Only articles pertaining to issues of conducting emergency research in developing countries mainly within the Middle Eastern region were included. Research papers published in languages other than English were excluded.

Table 1: Search Terms

Emergency research OR Emergency medicine	AND	Ethical issues OR Informed consent OR Emergency exception

Results

Considering the wealth of literature on emergency research and its related ethical issues in many developed countries, there was relatively little or no information about emergency research in the Middle East. Using the terms relating to emergency research and ethical issues (Table 1) 639 articles were elicited from all databases collectively. Combining these terms resulted in a total of 41 papers; after reviewing the titles and abstracts two were deemed to be totally unrelated for this review. Among the located studies eight were from Jordan [22-29], nine from Turkey[30-38], one from Yemen [39], five from Egypt[40-44], three from Iran[45-47], five from Lebanon[48-52], six from Saudi Arabia [53-58]and two from United Arab Emirates (UAE) [59, 60]. All located studies are summarized below in Table 2.

Since the mid of 90's, research concerning emergency medicine within emergency settings in the Middle East was conducted[34, 38]. Located studies focused on the development of emergency care specialty and services and the status of emergency medical care within different institutions[22, 36, 48]. In addition, several publications have highlighted the characteristics of emergency care visitors, appropriateness of emergency department visits and the level of satisfaction with provided care[23, 28, 30, 31, 33, 36, 37]. However, none was found on emergency research and ethical issues associated with it. Unfortunately, studies were not designed to investigate emergency research in any depth, but to describe technical aspects of available emergency medicine and offered practices and/or services. It appears as to confront a situation with major ignorance of scientific emergency research and its associated ethical issues within emergency settings in the Middle East.

Despite the fact that, ample research has been conducted within our region to discuss aspects of ethical considerations in different medical departments[61-64]. None was found covering such considerations within the field of emergency research. Such results reflect the dearth of evidence on conducting emergency research within the Middle Eastern

Table 2: Summary of located papers covering issues related to emergency medicine practice and research in the Middle-East region

Study (year), ref no	Study design	Country	Study setting	Study sample	Main findings
(1995), 38	Descriptive with recommendations	Turkey	Descriptive	NA	Emergency medical care in Turkey. Future directions
(1995), 34	Cross-sectional, retrospective	Turkey	ED records over one year, from one hospital	NA	Common causes for admission were infectious, respiratory, and neurological diseases. Infectious diseases were the most common cause of mortality.

Study (year), ref no	Study design	Country	Study setting	Study sample	Main findings
(1996), 48	Descriptive with recommendations	Lebanon	Descriptive with recommendations	NA	Startup of education and training programs for health care workers was needed to produce a comprehensive plan for emergency care in Lebanon.
(1997), 22	Descriptive with recommendations	Jordan	Descriptive	NA	Efforts were recommended to extend the EMT programs in Jordan.
(2000), 24	Cross-sectional, retrospective	Jordan	ED records over one month, from one hospital	2841 patients' records	Inappropriate use of emergency services was found to be very common
(2000), 58	Perspective study by external ED physician	KSA	NA	NA	Lack of emergency medicine training programs in the Middle East, so the ED staff is largely expatriate.
(2001), 36	Descriptive with recommendations	Turkey	Descriptive	NA	Developing international collaboration in the Middle East region to promote emergency medicine specialist in the national emergency care system.
(2003), 28	Cross-sectional, retrospective	Jordan	ED records over 6 months, from one hospital	29463 patients' records	Frequent non-urgent ED visits adversely affect the quality of provided care and patients' satisfaction
(2003), 33	Cross-sectional, prospective	Turkey	Hospital-based survey in the ED at one hospital over 2 weeks	1155 patients	High rate of inappropriate ED services usage
(2004), 30	Cross-sectional, prospective	Turkey	Hospital-based survey in the ED at one hospital over 2 weeks	1113 patients	healthcare providers and the hospital characteristics had the greatest impact on overall satisfaction of ED patients
(2005), 31	Cross-sectional, prospective	Turkey	Hospital-based survey in the ED at one hospital over 6 months	245 patients	Good patient satisfaction was related to high quality care and insurance restrictions, while lengthy waiting time was the main reason of dissatisfaction

Study (year), ref no	Study design	Country	Study setting	Study sample	Main findings
(2006), 37	Cross-sectional, retrospective	Turkey	ED records over 6 months, from one hospital	3422 patients' records	Acute abdomen (bleeding) was the most common reason for emergency surgery
(2007), 23	Cross-sectional, retrospective	Jordan	ED records over one year, from one hospital	73259 patients' records	Most common causes of ED visits (e.g. chest pain and CVS disorders, SOB and RS disorders, abdominal pain and GI disorders)
(2009), 27	Cross-sectional, prospective	Jordan	Hospital-based survey in the ED at one hospital over 2 weeks	4592 patients	Good patient satisfaction was indicated with some complaints on long waiting time and insufficient staff and beds
(2009), 59	Descriptive with recommendations	UAE	NA	NA	The development of a regional or national program for accreditation in emergency medicine will be important
(2010), 26	Cross-sectional, retrospective	Jordan	ED records over one month, from one hospital	4950 patients' records	Inappropriate use of emergency services was found very common by non urgent cases.
(2010), 32	Cross-sectional, prospective	Turkey	ED records over 2 months, from one hospital	2079 patients	Number of ED admissions and clinical features of ED patients were not different during the month of Ramadan
(2011), 47	Cross-sectional, prospective	Iran	Hospital-based survey in the ED at one hospital over one week	500 patients	Efforts on shortening waiting time and improving patients' perceptions about waiting in ED, as well as improving the overall cleanliness of the emergency room are highly recommended.
(2012), 35	Cross-sectional, retrospective	Turkey	ED records over one month, from one hospital	21014 patients	Approaching one quarter of ED visits were inappropriate, where inappropriate use of ED resources was more frequent during specific shifts.
(2013), 25	Official letter with recommendations	Jordan	ED records 2006-2011	Patients' records over 6 years	Supportive of the involvement of GP and FM in ED practice

Study (year), ref no	Study design	Country	Study setting	Study sample	Main findings
(2013),45	Cross-sectional, prospective Validation study	Iran	Hospital-based survey in the ED at one hospital over 5 months	1104 pediatric patients ESI tool used by pediatric trained nurses (12) and physicians (4)	Admission rate increased as the ESI score decreased.
(2013), 50	Descriptive with recommendations	Lebanon	Description of history of prehospital emergency care development with description of existing services.	NA	Emergency medical status is related to limited human resources, insufficient technical resources, scarce high-level management and system-thinking leadership, and gaps in public education efforts.
(2013),46	Cross-sectional, retrospective	Iran	ED records over one month, from one hospital	1923 patients ED visits	Inappropriate ED visits was associated with evening and night shifts, payment by health insurance, and the ED visit reason was to obtain rapid treatment
(2013), 40	Descriptive with recommendations	Egypt	Descriptive with recommendations	NA	Emergency medicine as specialty suffered from unqualified personnel from other specialities and lacking of resources
(2014), 49	Cross-sectional, prospective	Lebanon	Hospital-based survey in the ED at all ED departments hospital over 6 months	62 managers and/or directors of ED	Noninvasive positive pressure ventilation is more frequently used in EDs in private hospitals.
(2014), 60	Descriptive with recommendations	UAE	NA	NA	Creation of the Emirates Society of Emergency Medicine, the availability of residency training sites, and the development of fellowship programs, will advance emergency medicine specialty
(2014), 57	Cross-sectional, prospective	KSA vs. USA	Online survey for emergency programs at multiple hospitals in KSA and USA over 2 months	73 emergency residents	The Saudi residents see more patients per hour compared to US peers. Saudi trainees felt less competent in less common procedures than US trainees. In KSA, compared to USA, less formal didactics and simulation experience emergency training was evident.

Study (year), ref no	Study design	Country	Study setting	Study sample	Main findings
(2015), 29	Cross-sectional, prospective	Jordan	Hospital-based survey in the ED at one hospital over one year	63 healthcare providers	Good healthcare providers' satisfaction about the implemented morning report in clinical management of ED cases
(2015), 41	Cross-sectional, prospective	Egypt	Hospital-based survey in one hospital over one month	128 Emergency nurses 147 non-emergency nurses	Nurses working in emergency hospital experienced a higher level of different types of workplace violence
(2015), 56	Cross-sectional, prospective Validation study	KSA	Hospital-based case scenario assessment at one tertiary-care-center	10 emergency nurses	The Canadian Emergency Department Triage and Acuity Scale has good reliability among emergency department
(2016), 43	Cross-sectional, prospective	Egypt	Hospital-based survey in the ED at one hospital over 6 months	266 and 284 emergency physicians and nurses, respectively	About one-quarter of study nurses and physicians suffered from high levels of burnout syndrome.
(2017), 53	Cross-sectional, retrospective	KSA	Hospital-based interview in ED at 6 centers over 5 months	437548 patient ED visits	Low levels of readiness of EDs in academic hospitals to manage pediatric patients with critical missing components needed for pediatric emergency care
(2017), 54	Cross-sectional, prospective	KSA	Hospital-based survey in the ED at one hospital over 6 months	250 patients	Rapid emergency medicine score is beneficial for the risk stratification of patients present with chest pain to the EDs.
(2017), 55	Letter to the editor	KSA	NA	NA	High priority to identify challenges of EMT to allocate available resources with the goal of producing competent, Saudi-trained EMTs.
(2018), 39	Cross-sectional, prospective	Yemen	Hospital-based survey in the ED at one hospital over 2 weeks	531 healthcare providers	Insufficient knowledge of Yemeni health professionals regarding emergency and disaster preparedness.

Study (year), ref no	Study design	Country	Study setting	Study sample	Main findings
(2018), 42	Cross-sectional, prospective	Egypt	Hospital-based survey in the ED at one hospital over 4 months	149 patients	Transport time for patients from injury to hospital arrival was around 4 hours, and mean ambulance response time was 45 minutes.
(2018), 44	Cross-sectional, prospective	Egypt	Hospital-based survey in the ED at one hospital over 3 months	108 emergency physicians	Most of the physicians serviced on average 20-40 patients per shift, and worked for 40–60 working hours per week. Thus, their Professional quality of life was affected.
(2018), 51	Cross-sectional, retrospective	Lebanon	ED records over one year, from one tertiary-care-center	12,637 pediatric E D visits	Fever of unknown origin, external injuries, upper RS infections, open wounds, and abdominal pain were the most common reasons for pediatrics ED visits
(2018), 52	Cross-sectional, retrospective	Lebanon	ED records over 6 years, from one	108 patients	ED visits by suicidal attempters were carried out predominantly at home, on a weekday, and by using overdose on prescription drugs.

Abbreviations:
NA: Not applicable or not available; ED: Emergency Department; CVS: Cardiovascular system; SOB: Shortness of breath; RS: Respiratory system; GI: Gastrointestinal; GP: General practitioner; FM: Family medicine; EMT: Emergency medicine training; KSA: Kingdom of Saudi Arabia; US: United States; UAE: United Arab Emirates.

Discussion

Emergency research is essentially important and necessary to enhance and improve public health related outcomes. In the Middle East, emergency settings are crowded by several endemics and outbreaks [65].Therefore, it is important to highlight ethical issues related to emergency research in such setting.

Based on the findings of our literature review, data related to emergency medicine research and ethical governors in MENA region is scarce, and research field in emergency settings is a shady, mysterious area. A potential explanation to this finding is lack of enforced regulations related to emergency research, and disciplinary rules as reported in the review of national research ethics guidelines in Arabic Middle Eastern countries conducted by Alahmad and colleagues [39]. There was a lack of regulations related to research ethics in vulnerable persons except in Egypt and Qatar where this area was covered in local regulations of both countries. In general, local research highlights the needs for more clinical-research and bioethics focused training, which might be initiated by the development and enhancement of educational approaches for healthcare professional students [66]. Additionally, the problem of medication shortage [67] and low doctor to patient ratio at emergency departments compared to the acceptable ratio as promulgated by WHO, and overcrowded emergency departments in developing countries [39] rendering such setting as less favorable environment for research despite its highly dynamic nature and the vast potential for research conduction.

Furthermore, lack of social awareness, false beliefs related to the concept of research, limited research funding, as well as misunderstanding of research-related terms such as informed consent and legal guardian could

further restrict research in developing countries in general, and in incapacitated emergency departments' patients in particular[39].

Another confounder related to emergency research is the potential conflict related to legal surrogate in light of the absence of the term (durable power of attorney) in MENA region. This aspect is partially governed by local cultural issues and lack of advance care planning in low-income MENA countries[39]. As dictated by cultural beliefs, the role of surrogate is assigned by default to the spouse, older son of the family, or to the older brother based on the depth of familial relationship and inferences.

The strength of the present review lies in covering an important overlooked field that has not been covered by other research conducted in the MENA region. Furthermore, extensive literature review and rigorous search of pertinent studies was followed to prepare this review. As aforementioned, different engines were searched using different search terms. This adds to the reliability of our results. Likewise, it is important to report the possible limitations. Firstly, the limited number of published papers in the scope of emergency research in the MENA region could hinder the accountability of the review. Besides, exclusion of non-English papers is another potential limitation.

The present review is expected to impact the current practices related to emergency research. Reported deficiencies in the area of emergency research should alarm the regulatory authorities to set some rules to better outline research conducted in emergency settings. Additionally, educational campaigns targeting general population should be reinforced to raise awareness level related to responsible conduct of research, and to stress the importance of research in saving lives of patients and their relatives. Future research assessing population and emergency care providers' perception towards research and related terms, including informed consent is recommended in MENA region to better spot barriers compromising research as perceived by emergency healthcare providers and general population.

Conclusion

The present review highlighted the lack of contemporary data to fully appraise emergency research in the Middle East. Any conducted research in this field is based upon tenuous evidence along vague rules, and this underlines the importance of enforced regulations to protect the welfare of general population and feature scientific emergency services and practices. Noteworthy, Middle Eastern region is geographically close to European countries, which have well-established rules and regulations related to research within emergency settings. As guided by political instability, the Middle East has flooded a significant percentage of refugees to neighboring developed countries, including Europe. The present review highlight the imminent needs to prepare and enact firm rules and regulations better regulate all aspects of medical experiments on human subjects, including emergency research in the Middle East. As a future direction, alignment of consolidated rules and regulations that govern emergency research in the Middle East should improve implemented services and practices within emergency settings in different countries.

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وضع البحث في حالات الطوارئ في منطقة الشرق الأوسط

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ملخص

يعد إجراء البحوث العلمية في حالات الطوارئ أمرًا بالغ الأهمية لتعزيز المعرفة والخبرات حول التعامل مع المرضى ذوي الحالات الحرجة والطارئة. ومع ذلك، فإن إشراك هؤلاء المرضى، والذين هم في الغالب غير قادرين على الموافقة والمشاركة في البحث، يمثل تحديًا كبيرا للباحثين ولمزودي الرعاية الصحية. تصف هذه الورقة البحثية الوضع العام للأبحاث في حالات الطوارئ والقضايا الأخلاقية ذات الصلة في منطقة الشرق الأوسط. حيث تم إجراء مراجعة شاملة للعديد من قواعد البيانات وقام الباحثين بتحديد العديد من الدراسات التي أجريت داخل أقسام الطوارئ في منطقة الشرق الأوسط. ركزت جميع الدراسات المنشورة على انماط توفير الرعاية الطارئة، بينما لم يصف أي منها جوانب الأبحاث العلمية في حالات الطوارئ. تشير الأبحاث العلمية الحالية إلى ندرة الدراسات المعنية بظروف البحث في حالات الطوارئ في البلدان النامية بشكل عام وفي منطقة الشرق الأوسط بشكل خاص. تعتبر هذه الدراسة الأولى من نوعها لمراجعة الأبحاث المتوفرة حول طبيعة أبحاث الطوارئ في الشرق الأوسط. تشير النتائج المستخلصة من هذه المراجعة إلى أهمية تشجيع وتحفيز المزيد من الجهود التي تركز على أبحاث الطوارئ في المنطقة، من أجل تحسين فعالية وسلامة توفير الرعاية الصحية لمرضى الحالات الحرجة داخل أقسام الطوارئ. إضافة إلى ذلك، تعبر نتائج هذه الدراسة عن الحاجة لهيئات ومؤسسات تنظيمية تساعد في التغلب على القضايا الأخلاقية الرئيسية المتعلقة بمبدأ الحصول على موافقات مستنيرة للمشاركة في الأبحاث المعنية بحالات الطوارئ.

الكلمات الدالة: حالات طوارئ، أبحاث، أخلاقيات، منطقة الشرق الأوسط .

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Quality of Life in Disabled Versus Able-Bodied Individuals during COVID-19 Pandemic

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ABSTRACT

Background: COVID-19 pandemic emerged in China, Wuhan in December, 2019. This pandemic has affected most domains of quality of life (QoL) for all individuals.

Objective: The aim of this study was to assess the quality of life among disabled persons and healthy-normal individuals during COVID-19 pandemic to compare it with their QoL before COVID-19 pandemic, in Jordan.

Methods: Six hundred and thirty nine able-bodied participants (33.8 ± 11.3 years) and 143 disabled individuals (46.8 ± 16.4 years) completed the WHOQOL-BREF (a tool used to measure Quality of life) which is consisted of 24 items distributed in four domains (physical health, psychology, social relationships and environment) and 2 items on overall quality of life and general health. The survey was distributed to participants online through social media (WhatsApp, Facebook, emails) between 12th June and 18th July 2021.

Results: Quality of life values were higher in able-bodied participants for physical health (65.5 ± 16.3 vs. 56.2 ± 19.8), social relationships 63.2 ± 19.7 vs. 55.3 ± 21.1) and environment (53.6 ± 16.6 vs. 49.8 ± 17.9) domains. The quality of life correlated positively with individuals' income for both groups and higher in all domains for physically active compared to non-physically active participants. Screen time significantly increased during COVID-19 for both groups.

Conclusion: The authors recommended that more attention should be paid to all items of quality of life during COVID-19, particularly with regard to disabled persons, and to potential deleterious effects which may result from sedentary lifestyle behavior such as higher screen time usage during COVID-19.

Keywords: Quality of life; able-bodied; disabled; COVID-19 pandemic.

1. INTRODUCTION

Coronavirus Disease (COVID-19) is a global pandemic, which first appeared in December 2019 in Wuhan, China, when cases of pneumonia of unknown etiology were reported [1]. On 11 March 2020 The World Health Organization (WHO) declared COVID-19 a pandemic. To date, there have been 261,435,768 people infected by COVID-19 and 5,207,634 have died as a result of the infection worldwide [2, 3]. In Jordan, approximately 953,943 people have been reported to be infected by COVID-19, and 11,608 deaths [4].

The World Health Organization (WHO) quality of life[5] defined QoL as the Individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns. QoL is affected by the persons' life goals, expectations, standards, and concerns [6, 7]. QoL assessment is increasingly used to

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describe subjective well-being in population studies as well as intervention outcomes in clinical trials [8]. WHOQoL-100 and its abbreviated version WHOQoL-BREF have been developed under the leadership of WHO over the past three decades [6, 7]. The QoL-BREF is commonly used to assess QoL since it is relatively short (26 items), convenient to use and valid across cultures. The QoL-BREF measures four domains physical health, psychology, social relationships and environment. There are two questions of QoL-BREF assesses general health and overall quality of life.

Most countries worldwide have adopted many restrictive policy measures to contain the transmission and diminish the spread of COVID-19 such as closing schools, restricting populations to their homes with permission to leave only for necessary work, shopping, or medical reasons, using facial mask, social distancing and prevention of large gatherings [9, 10, 11]. Such policy measures have affected most sectors. For example, in the USA, the unemployment rate rose from 3.8% in February 2020 to 14.7% in April 2020 with 23.1 million are unemployed [12]. These implemented policy measures have also affected the quality of life. For example, [13] observed a significant worsening of health-related quality of life in the entire sample of older adults (76.24 ± 6 years) in Spain. In addition, [14] reported that COVID-19 has affected the QOL for both Saudi and non-Saudi between 18-65 years old and this effect was more severe among individuals with medical conditions and those who lost their jobs due to COVID-19.

To the best of our knowledge, no studies have assessed the relations between COVID-19 pandemic related restrictive measures and the quality of life among disabled individuals compared to their able-bodied peers, in Jordan. Therefore, the aim of this study was to assess the quality of life among disabled persons compared to healthy-normal individuals during COVID-19 pandemic vs. before COVID-19 pandemic. A secondary aim was to assess whether quality of life was modified by gender, physical activity and educational level. We hypothesized that quality of life will be affected negatively during COVID-19 compared to before COVID-19 pandemic for both groups. It was also hypothesized that the negative effect of COVID-19 on quality of life would be higher for disabled individuals than able-bodied peers. Furthermore, it was hypothesized that quality of life would be affected positively by physical activity, income and educational level.

2. Material and methods

2.1 Participants

Data were collected from 782 Jordanian individuals (able-bodied = 639 (81.7%); disabled = 143 (18.3%) to assess the impact of COVID-19 on the quality of life. The type of disabilities included in this study were physical disabilities (spinal cord injury, spina bifida, amputation and poliomyelitis) n = 112, hearing impairment, n= 16 and visual impairment, n = 15. The mean age for able-bodied participants and disabled individuals was 33.8 ± 11.3 years vs. 46.8 ± 16.4 years, respectively. An information sheet was available on the first page of the questionnaire. The participants were free to withdraw at any time without giving explanations and no personal identification was requested to retain information confidentiality. No personal identification was requested to maintain privacy and anonymity, participants were also informed on their right to withdraw freely at any time without giving explanations. The inclusion criteria for the study were; 1) ≥ 18 years old, 2) Jordanian citizen; 3) provision of written consent after reading the aims of the study. 52.0% of able-bodied participants and 60.8% of disabled persons were married. Most able-bodied participants had completed a bachelor degree or postgraduate studies (532 (83.3%)) compared to (33(23.1%)) of disabled individuals.

2.2 Procedures

The WHOQOL-BREF survey was uploaded and shared on the Google online survey platform. A link to the electronic survey was distributed via social networks (e.g., Facebook, Instagram), e-mails, and messaging groups

(e.g., WhatsApp) during the period 12th June 2021–18th July 2021 using a snowball sampling strategy, since face-to-face contact was not possible for all individuals due to the COVID-19 pandemic. In order to avoid duplicate responses, each participant was allowed one response using Google form restrict options. The survey was distributed to Jordanian individuals who live in Jordan and were asked to fill in the survey. The information about the objectives of the study was provided and informed consent was requested. The participants were also informed that they would not be paid for participation in the study. The Institutional ethics approval was obtained by the school of Sport Sciences at the University of Jordan.

2.3 Instruments

The WHOQoL-BREF (26 items) was developed to provide a short form quality of life assessment [6] . The total of 26 original items were divided into: two items on overall quality of life and general health. The remaining 24 items, were classified into four domains; physical health (7 items), psychological (6 items), social relationships (3 items) and environment (8 items). The Arabic version of this form was used in the current study to assess the quality of life for both groups [6]. The translated version was checked by three of the academic staff members at the School of Sport Sciences at the University of Jordan and was translated back to the English language to ensure the accuracy and suitability of the form. The participants rated their quality of life based on a five-point scale (1, 2, 3, 4 and 5). The words corresponding to each number were (very poor, poor, average, good, and very good). The reliability values of the WHOQoL-BREF using Cronbach's alpha to assess internal consistency are shown in **Table 1.** The participants were asked to answer all questions. If one item of physical health, psychological and environmental domains was coded missing the means of each domain for each participant was substituted. Three items were reversed before scoring (i.e., B3, B4 and B26). Each raw score was transferred to 0-100 scale using this formula [(actual raw score – lowest possible raw score)/possible raw score range]*100. Possible raw score range is the difference between maximum and lowest possible raw score. For example, in physical health domain the lowest possible raw score is 7 and possible raw score range is 28 (maximum possible raw score (35) – lowest possible raw score (7) =28). Therefore, if the actual raw score of one participant was 12 in the physical health domain, this value transferred to be 28.6 [(15-7)/28)]*100 = 28.6.

The social relationships domain consists of 3 items. However, item number B21(How satisfied are you with your sex life?) was answered by married individuals only, as related to Jordanian religious and cultural values this item is not applicable to unmarried individuals (i.e., single, divorced and widowed).

Therefore, this domain was analyzed in two ways. First, social relationships for all participants (able-bodied n=639 and disabled n=143) which consisted of two items (B20 and B22) and second, social relationship for married participants (able-bodied n=322 and disabled n=87) which consisted of 3 items (B20, B21 and B22).[15] reported that 8.8% of 1052 participants did not answer item B21.

Table 1: Cronbach's alpha values of reliability for each domain and all questions for both groups

	Physical health	Psychological	Social relationships	Environment	All items
Able-bodied	0.784	0.787	0.693 (332)	0.814	0.922
Disabled	0.863	0.814	0.736 (87)	0.864	0.954

2.4 Data Analysis

Statistical analysis was conducted using the IBM SPSS (Statistical Package for the Social Sciences) software version 16.0. Mainly, means, standard deviation and

percentages were used. Spearman's correlation coefficient was used to assess whether there was a significant association between monthly income and all domains of quality of life for able-bodied and disabled persons. A series of independent sample t-test were used to compare whether there was a significant difference in the mean scores of all domains of quality of life between able-bodied and disabled individuals, males and females, physically active and non-physically active and between single and married individuals. In addition, a series of analysis of variance (ANOVA) were used to compare whether there was a significant difference in the mean scores of all domains of quality of life during compared to before COVID-19 between the levels of education. Levene's test was used to check homogeneity of variance in t-test and ANOVA and if this assumption was violated, the degrees of freedom were adjusted. McNamara's test was used to compare whether there was a significant difference in screen time usage during compared to before COVID-19 for able-bodied and disabled individuals.

Results

All demographic information of the study sample and mean score of all domains of quality of life are shown in **Table 2**.

Table 2: Participants' sociodemographic characteristics and QoL score for each domain. Values are mean ± standard deviation.

		n	Physical health	Psychological	Social relationships	Environment	Social relationships for married
Gender							
Able-bodied	**Male**	255	68 ± 16	61 ± 16	65 ± 21	53 ± 17	64 ± 19 (139)
	Female	384	64 ± 17	58 ± 16	63 ± 22	54 ± 17	62 ± 21 (193)
Disabled	**Male**	118	56 ± 20	61 ± 18	60 ± 21	49 ± 18	55 ± 21 (80)
	Female	25	58 ± 21	59 ± 18	58 ± 25	53 ± 15	54 ± 25 (7)
Education level							
Able-bodied	**Less than High School**	15	55 ± 19	50 ± 16	53 ± 28	40 ± 18	46 ± 22 (13)
	High School	48	63 ± 17	56 ± 18	60 ± 25	48 ± 18	59 ± 24 (31)
	Diploma	44	63 ± 14	56 ± 18	62 ± 20	50 ± 17	65 ± 19 (34)
	Bachelor	372	66 ± 16	59 ± 16	65 ± 22	54 ± 16	65 ± 19 (160)
	Postgraduate	160	67 ± 16	62 ± 17	66 ± 21	56 ± 16	64 ± 20 (94)
Disabled	**Less than High School**	52	49 ± 18	54 ± 17	54 ± 19	46 ± 16	47 ± 18 (39)
	High School	48	62 ± 20	65 ± 16	65 ± 20	52 ± 17	63 ± 12 (31)
	Diploma	10	48 ± 23	57 ± 13	50 ± 26	48 ± 17	44 ± 31 (7)
	Bachelor	22	55 ± 18	61 ± 19	63 ± 25	50 ± 22	63 ± 37 (5)
	Postgraduate	11	73 ± 16	69 ± 19	65 ± 27	63 ± 18	75 ± 29 (5)

Living Place							
Able-bodied	**City**	541	66 ± 16	60 ± 16	65 ± 21	55 ± 16	64 ± 20 (279)
	Village	86	60 ± 17	55 ± 19	61 ± 25	48 ± 19	58 ± 22 (47)
	Camp	12	64 ± 20	61 ± 14	65 ± 25	41 ± 15	75 ± 0 (6)
Disabled N	**City**	64	62 ± 19	63 ± 18	62 ± 26	52 ± 19	59 ± 27 (28)
	Village	74	51 ± 20	59 ± 18	57 ± 18	48 ± 17	54 ± 18 (56)
	Camp	5	53 ± 14	53 ± 4	63 ± 20	51 ± 11	56 ± 13 (3)
Income							
Able-bodied	**Less than 500 jd**	302	63 ± 16	56 ± 17	61 ± 23	48 ± 17	58 ± 21 (106)
	From 500 -799 jd	163	67 ± 16	61 ± 16	66 ± 21	55 ± 15	66 ± 20 (102)
	From 800 -1000 jd	73	67 ± 17	64 ± 14	67 ± 18	62 ± 13	65 ± 16 (53)
	More than 1000 jd	101	70 ± 15	65 ± 15	66± 21	63 ± 15	66 ± 19 (71)
Disabled	**Less than 500 jd**	120	54 ± 20	59 ± 18	58 ± 21	48 ± 18	52 ± 19 (73)
	From 500 -799 jd	17	66 ± 16	67 ± 12.49	68 ± 20	57 ± 19	70 ± 16 (10)
	From 800 -1000 jd	3	74 ± 9	71 ± 11	75± 22	63 ± 3	88 ± 6 (2)
	More than 1000 jd	3	70 ± 25	68 ± 20	54 ± 44	60 ± 18	54 ± 65 (2)
Are you current ill							
Able-bodied	**Yes**	56	48 ± 17	53 ± 18	55 ± 25	49 ±19	53 ± 22 (36)
	no	583	67 ± 15	60 ± 16	65 ± 21	54 ± 17	64 ± 19 (296)
Disabled	**Yes**	76	49 ± 18	58 ± 17	55 ± 17	47 ± 17	53 ± 16 (57)
	no	67	64 ±19	64 ± 18	64 ± 25	53 ± 18	59 ± 26 (30)
Physically activity							
Able-bodied	**Yes**	391	68 ± 15	62 ± 16	66 ± 21	55 ± 17	66 ± 19 (182)
	no	248	61 ± 17	55 ± 17	61 ± 22	52 ± 16	60 ± 21 (150)
Disabled	**Yes**	84	65 ± 16	67 ± 15	67 ±20	55 ± 16	62 ± 18 (49)
	no	59	44 ± 19	52 ± 17	50 ± 21	42 ± 18	47 ± 22 (38)

Gender

For able-bodied participants, independent samples t-test results showed that males have significantly better quality of life compared to females in physical health and psychological domains, $t^{(637)} = 2.887$, $P = 0.004$ and $t^{(637)} = 2.614$, $P = 0.009$, respectively. However, no significant differences between males and females in domain social relationships for married, environment and domain social relationships for all participants, $P > 0.05$. For disabled participants, t-test results showed no significant differences were observed between males and females in all domains of quality of life ($P > 0.05$).

Physical activity

For able-bodied participants, independent samples t-test results showed that physically active participants reported significantly better quality of life compared to non-physically active participants, $t^{(637)} = 5.273$, $P = 0.000$; $t^{(637)} = 5.415$, $P = 0.000$; $t^{(330)} = 2.608$, $P = 0.010$; t

$^{(637)}$ = 2.344, P = 0.019; t $^{(637)}$ = 2.954, P = 0.003 for the domains of physical health, psychology, social relationships for married, environment and social relationships for all participants domains, respectively. For disabled participants, independent samples t-test results showed that physically active participants reported significantly better quality of life compared to non-physically active participants, t $^{(141)}$ = 6.936, P = 0.000; t $^{(141)}$ = 5.494, P = 0.000; t $^{(85)}$ = 3.757, P = 0.000; t $^{(141)}$ = 4.727, P = 0.000; t $^{(141)}$ = 4.965, P = 0.000 for physical health, psychology, social relationships for married, environment and social relationships for all participants domains of quality of life, respectively.

Monthly income

For able-bodied participants, there was a significant positive relationship between monthly income and physical health, psychological, social relationships for married, social relationships for all participants and environmental domains and for all items of quality of life, r $^{(637)}$, 0.172, P = 0.000; r $^{(637)}$, 0.231, P = 0.000; r $^{(330)}$, 0.138, P = 0.012; r $^{(637)}$, 0.356, P = 0.000; r $^{(637)}$, 0.092, P = 0.020 and r $^{(637)}$, 0.277, P = 0.000, respectively.

For disabled participants, there was a significant relationship between monthly income and physical health, psychological, social relationships for married and environmental domains and for all items of quality of life, r $^{(141)}$, 0.284, P = 0.001, r $^{(141)}$, 0.184, P = 0.028, r $^{(85)}$, 0.336, P = 0.001, r $^{(141)}$, 0.232, P = 0.005 and r $^{(141)}$, 0.267, P = 0.001, respectively. However, this relationship was not significant between monthly income and social relationship for all participants domain r $^{(141)}$ = 0.163, P = 0.052.

Educational level

For able-bodied participants, a series of one way ANOVA revealed a significant difference between the categories of educational level in physical health domain F $^{(4, 634)}$ = 2.673, P = 0.031, psychological domain F $^{(4, 634)}$ = 2.940, P = 0.020, social relationship domain for married participants, F $^{(4, 327)}$ = 3.327, P = 0.011 and environmental domain F $^{(4, 634)}$ = 5.281, P = 0.000. Post hoc analysis using LSD showed that individuals with postgraduate and bachelor level reported significantly higher scores for quality of life compared to those with less than high school level in physical health domain, P = 0.007 and P = 0.014, respectively and psychological domain, P = 0.007 and P = 0.029, respectively. Participants with postgraduate level also reported significantly higher scores for quality of life compared to those with high school level, P = 0.038 in psychological domain. LSD showed that individuals with postgraduate, bachelor, diploma qualifications and high school level education reported significantly higher scores for quality of life in social relationship domain for married compared to those with less than high school level, P = 0.002, P = 0.001, P = 0.003 and P = 0.045, respectively. LSD also showed that individuals with postgraduate, bachelor and diploma level education reported significantly higher scores for quality of life in environmental domain compared to those with less than high school level education, P = 0.001, P = 0.001 and P = 0.046, respectively. However, one way ANOVA revealed no significant difference between the categories of educational level in social relationship domain for all participants F $^{(4, 634)}$ = 1.832, P = 0.121.

For disabled individuals, a series of one way ANOVA revealed a significant difference between the categories of educational level in physical health domain F $^{(4, 138)}$ = 5.580, P = 0.000, psychological domain F $^{(4, 138)}$ = 3.424, P = 0.011 and social relationships domain for married participants, F $^{(4, 82)}$ = 5.191, P = 0.001. In physical health domain, LSD showed that individuals with postgraduate level reported significantly higher quality of life scores compared to those with less than high school, diploma and bachelor, P = 0.000, 0.003 and 0.011, respectively and individuals with high school education reported higher quality of life than those with less than high school education and those with diploma P = 0.001 and 0.037, respectively. In psychological domain, LSD showed that individuals with postgraduate and high school level

reported higher quality of life compared to those with less than high school education, P = 0.011 and P = 0.002, respectively. In social relationships domain for married participants, LSD showed that individuals with postgraduate and high school level reported higher quality of life compared to those with less than high school education, P = 0.003 and P = 0.001, respectively, and compared to those with diploma, P = 0.008 and 0.019, respectively. However, one way ANOVA revealed no significant difference between the categories of educational level in social relationships domain for all participants $F^{(4, 138)} = 2.406$, P = 0.052 and domain 4 $F^{(4, 138)} = 2.265$, P = 0.065.

Able-bodied versus disabled

Independent sample t-test showed that able-bodied participants reported higher quality of life in overall quality of life and general health ($t^{(186.301)} = 3.623$, P = 0.000), physical health domain ($t^{(780)} = 5.868$, P = 0.000), social relationships domain for all participants ($t^{(780)} = 2.206$, P = 0.028), social relationships domain for married ($t^{(417)} = 3.236$, P = 0.001) and environment domain ($t^{(780)} = 2.390$, P = 0.017). However, no significant difference was observed in the psychology domain ($t^{(780)} = 0.683$, P = 0.495). The values of all domains of quality of life for both groups are presented in **table 3**.

Table 3: Mean score and SD of WHOQoL-BREF for both groups (Score range 0-100 for all domains except overall quality of life and general health).

	Overall quality of life and general health	Physical Health	Psychological	Social Relationships	Environment	Social Relationships Married
Able-bodied	3.76 ± 0.78	66 ± 16*	59 ± 16	64 ± 22*	54 ± 17*	63 ± 20*
Disabled	3.45 ± 0.96	56 ± 20	60 ± 18	60 ± 22	50 ± 18	55 ± 21
Total	3.70 ± 0.83	64 ± 18	60 ± 17	63 ± 22	53 ± 17	62 ± 20

* Significant difference between able-bodied and disabled participants

Screen time usage

For able-bodied participants, McNamara's test showed that participants spent significantly more time using screens including iPads, TVs, mobiles, or computers during the COVID-19 period compared to before the COVID-19 (χ^2 (15, n = 639) = 360.573, p < .001). For example, the daily rate of screen time usage for more than 5 hours increased from 13.6% to 42.7% and use for less than 1 hour decreased from 13.8% to 5.8%. Similar results were found for disabled participants (χ^2 (14, n = 143) = 61.80, p < .001). For example, the daily rate of screen time usage for more than 5 hours increased from 10.5% to 21.0% and use for less than 1 hour decreased from 42.0% to 25.9%. Screen time usage during and before COVID-19 for able-bodied and disabled participants are shown in Table 4.

Table 4: Participant screen time usage before and during COVID-19. Values are mean (SD).

	Able-bodied		Disabled	
Screen time usage (hours)	**Before covid-19 N (%)**	**During covid-19 N (%)**	**Before covid-19 N (%)**	**During covid-19 N (%)**
Less than 1 h	88 (13.8)	37 (5.8)	60 (42.0)	37 (25.9)
From 1h to < 2h	155 (24.3)	54 (8.5)	37 (25.9)	33 (23.1)
From 2h to < 3h	152 (23.8)	68 (10.6)	15 (10.5)	16 (11.2)
From 3h to < 4h	103 (16.1)	100 (15.6)	13 (9.1)	18 (12.6)
From 4h to 5h	54 (8.5)	107 (16.7)	3 (2.1)	9 (6.3)
More than 5h	87 (13.6)	273 (42.7)	15 (10.5)	30 (21.0)

Discussion

Able-bodied participants reported higher mean values of quality of life than disabled individuals in physical health (65.5 ± 16.3 vs. 56.2 ± 19.8), social relationships 63.2 ± 19.7 vs. 55.3 ± 21.1) and environment (53.6 ± 16.6 vs. 49.8 ± 17.9) domains and no significant difference was observed between the two groups in the psychological domain. The mean values of all domains of quality of life for able-bodied participants in the current study are less than those values of quality of life reported in previous studies [8, 16]. [8] reported that the mean values for physical health, psychological, social relationships and environment domains were 73.5, 70.6, 71.5 and 75.1, respectively among 866 Australian participants of 20 years and older. [16] Reported that the mean values for physical health, psychological, social relationships and environment domains were 69.2, 66.7, 63.1 and 58.5, respectively among 1046 Indonesian participants of 17 years and above. These mean values of quality of life for able-bodied participants are less than the norms of physical health (78.8), psychology (75.9), social relationships (72.3) and environment (71.2) domains [17]. The lower mean values of quality of life in all domains during vs. before the COVID-19 compared to previous studies and international norms maybe explained by the negative effect of COVID 19 on psychological well-being e.g, Morgül et al. [18], economics e.g., Bhosale, [19] and social relations and emotional wellbeing in adults e.g., Brooks et al. [20].

The study results showed that this negative effect of COVID 19 on physical health, social relationships and environment domains of quality of life was more prevalent in disabled individuals. These findings are in agreement with previous studies in normal circumstances. For example, [15] reported that the mean values for physical health, psychological, social relationships and environment domains were 67.5, 61.0, 63.8 and 51.9, respectively among healthy individuals compared to those with chronic diseases 60.7, 58.9, 61.1 and 52.5, respectively. [17] also reported that the mean values for physical health, psychological, social relationships and environment domains were 41.9, 52.3, 60.3 and 63.6, respectively among 100 participants with depressive symptoms. Furthermore, [21] reported that the mean values of the four domains of quality of life among 50 Brazilian participants with major depression were 42.9, 38.5, 41.3 and 42.9, respectively. This can be attributed to the fact that the disabled person has a greater problem in achieving a satisfactory quality of life since he/she has lost or did not have the physical capacity for the necessary responses to start and maintain the relationships, interactions and participation that healthy persons have [22,23] . Therefore, more attention and focus should be paid for individuals with disabilities and chronic diseases during the COVID 19 and in difficult times. For example, allowing disabled individuals to go for a walk or wheelchair propulsion as well as allowing food delivery, medication, and personal care for disabled persons during lockdown and similar situations.

For both groups, able-bodied and disabled, physically active participants reported higher values in all domains of quality of life compared to their non-physically active peers ($P < 0.05$). These findings are in agreement with

previous studies which reported that physical activity improves health-related quality of life [24, 25]. [25] Reported that greater leisure-time physical activity and less leisure-time sedentary behavior are correlated with better long term health related quality of life among 70 years old individuals in Spain. Similarly, [24] reported that physically active individuals of 60 years and older reported higher quality of life in all domains than those who were less physically active in the USA. [26] also reported that physically active older Australian women have better mental health compared to their sedentary peers. This improved quality of life related to physical activity can be attributed to the fact that physical activity reduces cardiovascular risk factors and prevents and manages chronic diseases [27] , prevents functional limitation [28] , lowers risk of falls [29] and lowers anxiety and depression [30]. Physical activity also improves self-efficacy which directly improves the quality of life [31] .

For both groups, able-bodied and disabled, the least mean values of all domains of quality of life level were observed among individuals with less than high school level education. These findings are in agreements with previous studies [32, 33, 34] .For example, [33] reported that Indian educated housewives reported higher quality of life for general health, physical health, psychological and social relationships domains than non-educated peers. Similarly, [15] indicated that there was a strong relationship between education level and the four domains of quality of life. These findings are expected as education is regarded as an essential determinant of quality of life [32, 33]. [32] indicated that at the individual level, increasing education level increases worker productivity and therefore results in better employment and income for the individuals. [15] also suggested that education plays a greater role in maintaining health and higher quality of life among mainland Chinese than wealth. However, [35] showed that education did not affect quality of life among disabled individuals.

For both groups, able-bodied and disabled persons, there was a significant relationship between individuals' income and quality of life. These finding are in agreement with [36,16,37] .These authors reported that Indonesian individuals with monthly income of 5000 Rupiah or more have better quality of life in all domains than their peers with less monthly income. [37] Reported that exercises and monthly income were factors that affect health related quality of life positively. These authors reported that newly diagnosed angina patients with a monthly income ≥5000 Yuan showed higher health related quality of life scores than those of patients with a monthly income < 5000 Yuan. The poor health related quality of life among patients with low income can be attributed to the fact that patients with lower income may have a limited ability to obtain effective treatments, which may worsen their clinical outcomes.

Screen time usage increased considerably for both groups during compared to before the COVID-19 pandemic. For example, the percentage of able-bodied and disabled participants' screen time usage of 5 hours or more increased from 13.6% to 42.7% and from 10.5% to 21% respectively. These findings are in agreement with previous studies which indicated that screen time usage increased during compared to before COVID-19 pandemic among children in Jordan [38], UK[18] and Spain and Italy [39] . This might be attributed to the fact that people tend to use smart phones, tablets and TV more during the lockdown and social distance measures implemented by Jordanian government to control the spread of COVID-19. In Canada, 60% of men and 66% of women reported an increase in their TV time and 63% of men and 69% of women reported an increase in their internet usage [40].

Conclusion

It was recommended that the Jordan government should pay more attention and concern to disabled individuals by allowing them to go for a walk or wheelchair propulsion during lockdown and similar situations. The Jordanian government should adopt long term strategies to lessen the side effects which may increase from the sedentary lifestyle behavior such as increase of screen time usage and performing less of physical activity.

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جودة الحياة لدى الأشخاص ذوي الإعاقة مقارنة بالأفراد الأصحاء خلال جائحة كورونا (COVID-19)

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ملخص

ظهرت جائحة كورونا (COVID-19) في ووهان في الصين، في ديسمبر 2019. وقد أثر هذا الوباء على معظم مجالات جودة الحياة (Quality of Life) لجميع الأفراد. هدفت هذه الدراسة الى تقييم جودة الحياة لدى الأشخاص ذوي الإعاقة والأفراد الأصحاء خلال جائحة كورونا (COVID-19) مقارنة مع قبل جائحة (COVID-19) في الأردن. أكمل ستمائة وتسعة وثلاثون مشاركًا من الأصحاء (33.8 ± 11.3 عامًا) و 143 مشاركًا من ذوي الإعاقة (46.8 ± 16.4 عامًا) مقياس جودة الحياة النسخة المختصر (WHOQOL-BREF)) والذي يتكون من 24 فقرة موزعة في أربعة مجالات (الصحة البدنية، الجانب النفسي، والعلاقات الاجتماعية، والبيئة) وفقرتين عن الجودة الشاملة للحياة والصحة العامة. تم توزيع االمقياس على المشاركين الكتروتيا من خلال وسائل التواصل الاجتماعي (WhatsApp، Facebook ، رسائل البريد الإلكتروني) في الفترة ما بين 12 حزيران و 18 تموز 2021. أشارت النتائج أن قيم جودة الحياة كانت أعلى لدى المشاركين الأصحاء مقارنة بالافراد الاعاقة فيما يتعلق بالصحة البدنية (65.5 ± 16.3 مقابل 56.2 ± 19.8) والعلاقات الاجتماعية 63.2 ± 19.7 مقابل 55.3 ± 21.1) ومجال البيئة (53.6 ± 16.6 مقابل 49.8 ± 17.9). وارتبطت جودة الحياة طرديا بدخل الأفراد لكلتا المجموعتين وأعلى في جميع المجالات بالنسبة للمشاركين النشطين بدنيًا مقارنة بالمشاركين غير النشطين بدنيًا. وأشارت النتائج أن إستخدام الشاشات زاد بشكل ملحوظ خلال COVID-19 لكلا المجموعتين. أوصى المؤلفون بإيلاء المزيد من الاهتمام لجميع عناصر جودة الحياة خلال COVID-19، لا سيما لدى الأشخاص ذوي الإعاقة، والآثار الضارة المحتملة التي قد تنجم عن الخمول البدني وزيادة استخدام الشاشات أثناء COVID -19.

الكلمات الدالة: جودة الحياة؛ الأشخاص الاصحاء؛ الأشخاص ذوي الإعاقة؛ جائحة كورونا.

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Schizophrenia: The Ambiguous Mechanism behind the Disorder

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ABSTRACT

Background: Schizophrenia is considered one of the top 10 conditions that cause disability worldwide. Regardless of its low prevalence, it negatively impacts the quality of life not only for patients but also for families and society. The present antipsychotic therapies provide relief only for the positive symptoms, but they do not improve the negative or cognitive symptoms of schizophrenia. Extensive research is being conducted to discover new medications that can treat or prevent the illness. This can only be achieved by fully understanding the underlying mechanism behind the illness.

Methods: Four hypotheses which explain the possible mechanisms that might be involved in the development of schizophrenia have been discussed in this review. The effect of vitamin D and iron deficiencies, infection, and paternal age on the development of schizophrenia in the offspring were also reviewed, in addition, to the demonstration some of the clinical studies and their outcomes.

Results: The exact cause of schizophrenia is still not fully known. The disease might develop as a result of neurotransmitter dysfunction, receptor hypofunction, environmental factors, or other factors which might play a role in the etiology and course of schizophrenia.

Conclusion: All these factors which might be involved in the development of the illness are required to be investigated in order to provide new hope for people suffering from schizophrenia. Numerous studies are in progress to find the exact pathophysiology of the disease, but despite such progress, there are still many questions are required to be answered in order to assist us in developing the appropriate therapy for treating the illness.

Keywords: Antipsychotic, disability, hypotheses, mechanisms, schizophrenia.

1. INTRODUCTION

The term schizophrenia which means split mind comes from Greek roots schizo (split) and phrene (mind) (1). It is a mental disorder wherein the person split from reality. Schizophrenia is often confused with dissociative identity disorder in which the patient has more than one identity or personality where these identities take part in the patient's life (2). The concept of the disease can be difficult to understand. Healthy individuals who are not suffering from schizophrenia have little idea about the illness. Schizophrenia is a chronic psychotic disorder that affects how people think, live, and see the world. It is characterized by three symptoms: positive symptoms like hallucination and delusion, negative symptoms like social withdrawal and reduce interest in everyday activities and cognitive symptoms including reduced attention and memory changes (3, 4). The severity of the disorder, signs, symptoms, and the effect of the disease on the patient's quality of life may vary among individuals (5). Schizophrenia generally starts to develop in late adolescence and early adulthood, it impacts both genders equally. However, men usually start to experience the symptoms earlier at the age of 15-25, whereas women tend

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to develop the symptoms at the age of 25-35 (6).

Schizophrenia has been around for a long time, but due to its nature, the exact statistic is difficult to be obtained. Approximately 1% of the population worldwide are suffering from the disease (7). It is estimated that the illness costs the society US$94 million to US$102 billion per annum in United States. It imposes a burden on the society which finds it difficult to provide support for those patients through family and social bonds (8). Most of the developing countries suffer from high rate of mental illnesses (9). The majority of patients and their families are not aware of the severity of the disorder; they find that this disease is a stigma and should be hidden from the society. As a consequence, many patients who suffer from schizophrenia refuse to get appropriate treatment in order to protect themselves from being stigmatised as a maniac by the community (10). The etiology and pathophysiology of schizophrenia remain unclear but numerous theories have been suggested to illustrate the possible mechanisms involved in the development of the illness. This review highlights some of these theories (Fig. 1) and demonstrates their point of view in explaining the underlying mechanism of schizophrenia.

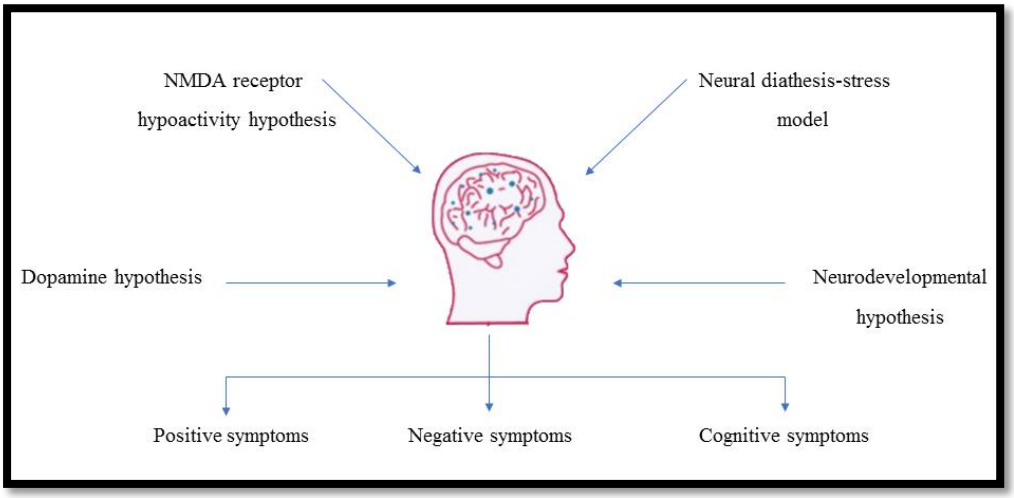

Fig. 1. Schizophrenia hypotheses. Some of the hypotheses that explain the possible mechanisms involved in the development of schizophrenia.

2. THE POSSIBLE UNDERLYING MECHANISMS INVOLVED IN THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA

2.1 Dopamine Hypothesis

This hypothesis is the oldest neurochemical theory of the pathophysiology of schizophrenia (11). It was proposed as a consequence of discovery that the antipsychotic drugs block the dopamine (DA) receptors in animal experiments (12). The usefulness of antipsychotic treatment was observed prior to the explanation of how it works. Studies later discovered the correlation between the clinical effectiveness and antipsychotics affinity for DA receptors (13). So far, the dopamine hypothesis provides the best explanation for the psychotic episode of schizophrenia; it suggests that the unusual behaviour and psychosis experienced by schizophrenic patient might be related to changes in DA level in the brain (14).

The early formulated hypothesis attributed the symptoms of schizophrenia to hyperactivity of DA transmission based on the observations that

psychostimulants activate the DA receptors and the important role of DA in the extrapyramidal motor system (15). The classical DA hypothesis was reformulated over the years, due to the increased awareness of the importance of persistent negative and cognitive symptoms in this disease and their resistance to antipsychotics. Brain imaging studies proposed that the patient develops the symptoms of schizophrenia due to the imbalance in DA in the brain (16). The positive symptoms arise as a result of over-activity of DA in subcortical mesolimbic pathway which augments D2 receptor activation in the brain. The DA hyperactivity can be attributed to either excessive release of DA from the presynaptic cell or pathological raise in D2 receptor on postsynaptic end (17). In addition, the positive symptoms might also develop due to the disturbance in the cortical pathway through the nucleus accumbens (18). On the other hand, the negative symptoms and cognitive impairment of schizophrenia result from the hypoactivity of mesocortical DA projections to the prefrontal cortex which leads to hypostimulation of D1 receptors in the brain (19, 20).

Recently, a comprehensive study conducted by researchers at Columbia University Irving Medical Center and New York State Psychiatric Institute found that people with schizophrenia who experience auditory hallucinations tend to hear what they expect. Those patients are known to have elevated levels of DA in the brain, where the elevation of DA could make some patients rely more on expectations, which could then result in hallucinations. Cassidy et al. have reported the dopamine-dependent mechanism that explains the reason of hallucination in psychotic patients. They induced auditory illusions in untreated patients with schizophrenia who experience varying degrees of hallucination and healthy volunteers to test a dopamine-dependent gain-control mechanism of hallucinations. They have requested the participants to judge the length of a target tone that preceded by context tones which were in three different conditions: shorter, same length, or longer than the target tone. The context tones were applied for the reason of setting up an expectation in the participants of hearing tones of a certain length, which biases subsequent perception of the target tone. Critically, as mentioned earlier, the influence of expectation on perception in health is dictated by how strong or reliable the expectation is. Cassidy and colleagues have manipulated this aspect by changing the variability of the context tones, in which all context tones were either the same length or fluctuated around the mean. The study results have reported that, in healthy participants, the influence of expectation on perception was modulated by this manipulation; the perceptual bias that was induced by the context tones was less strong in the condition with high variability compared to the one without. This finding suggests that when generating percepts, the auditory system of a healthy person weights the influence of expectation on perception according to its reliability. A different pattern emerged in untreated patients with schizophrenia: the higher the severity of hallucination, the stronger the biasing effect of expectation on perception; moreover, the reliability of the expectation had little or no modulatory influence on this bias. This finding indicates that, in schizophrenia, hallucination severity is associated with a stronger perceptual bias toward expected states and with failures to inhibit this perceptual bias in uncertain contexts. In order to study the DA role in this process, a subsample of participants was given a low dose of amphetamine, which develops the positive symptoms by increasing the DA levels in the nucleus accumbens. Participants, whose perception had previously been sensitive to the reliability of their expectation, became less sensitive after this pharmacological challenge due to the elevation in DA levels. Thus, the more DA a participant's brain generated, the less their auditory system down-weighted the influence of expectation when its reliability was low. These findings propose that increased DA levels lead to an overestimation of the reliability of expectation. This process disturbs the flexible integration of expectations into perceptual

experience, which might ultimately lead to hallucinatory percepts (21).

The typical and atypical antipsychotics have proven to be the best available treatment for schizophrenia so far. However, the treatment of the disease with antipsychotics is not definitive due to the fact that these medications treat only the positive symptoms by blocking the D2 receptors (Fig. 2), without improving the negative symptoms or cognitive impairments of the disorder (22). Further, typical antipsychotics are commonly cause extrapyramidal side effects due to excessively blockage of D2 receptors resulting in either DA blockade or depletion in basal ganglia (23, 24). To date, no therapy has been found for alleviating the negative symptoms and cognitive impairments of schizophrenia. Thus there is an urgent unmet medical need, which must be fulfilled by discovering newer therapies (25).

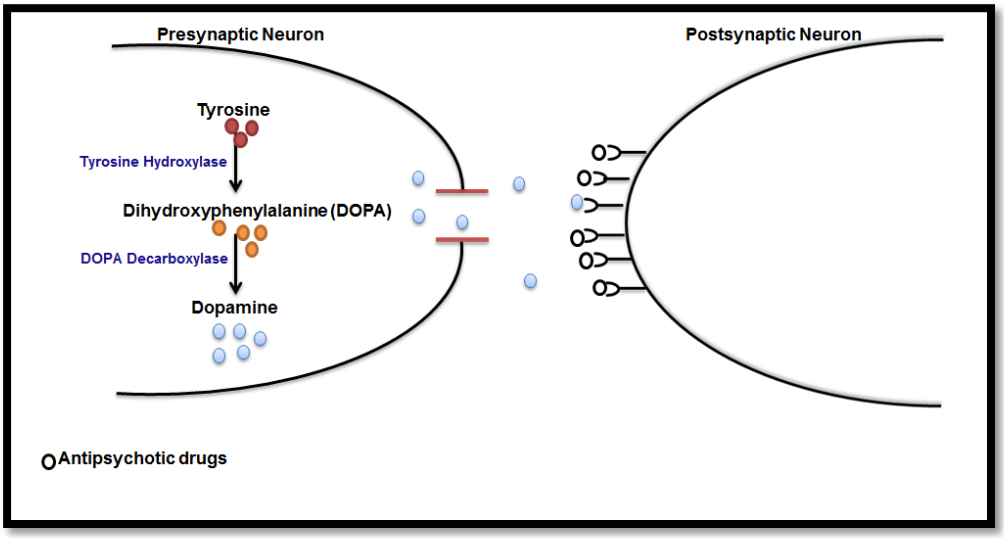

Fig. 2. Antipsychotic drugs and dopamine compete for the same receptor sites. The principle mechanism of action of typical and atypical antipsychotics is activation of indirect pathway by blocking D2 receptors.

2.2 The N-methyl-D-aspartate Receptor Hypoactivity Hypothesis

N-methyl-D-aspartate (NMDA) receptors belong to the glutamate receptor family; they are considered the most important excitatory neurotransmitter receptors in the brain. The expression and regulation of NMDA receptors properly in the brain are crucial for cortical plasticity, maturation, learning and memory processes. This hypothesis postulates that NMDA receptor hypofunction, which is a condition that induced in human or animal brain by using NMDA receptor antagonist, might be viewed as a model for a disease mechanism, where the course of schizophrenia may be attributed to either dysfunction or blocking of the NMDA receptors (26, 27). Glutamate and acetylcholine which are excitatory neurotransmitters in the brain are excessively released in the cerebral cortex as a consequence of NMDA receptors blockage. Studies reported that, the cognitive and behavioural disturbances associated with NMDA receptor hypofunction explained by the excessive release of excitatory neurotransmitters and overstimulation of postsynaptic neurons (28). It has been assumed that both genetic and environmental factors can participate in the NMDA receptor hypofunction condition where this condition established in the brain early in life as a latent state which trigger psychotic manifestation during the adulthood life but not before that because, the pathological potential

can be expressed after the occurrence of certain maturational changes in the circuitry of the brain. When these maturational changes have taken place, the NMDA receptor hypofunction has the potential to trigger the symptoms of schizophrenia and in advance cases, to cause ongoing structural deterioration (26).

In the past two decades, evidences from both human subjects and animal models revealed that different aspects of molecular, cellular, and behavioural abnormalities related to schizophrenia are due to the aberration in the function of NMDA receptor in the limbic region of the brain (29). The NMDA hypothesis was based on the experimental observation, in which the NMDA receptor antagonists such as phencyclidine (PCP) and ketamine can produce schizophrenia-like symptoms in healthy people (30). Evidence has suggested that the intoxication with PCP can trigger the positive and negative symptoms of schizophrenia. In addition, the PCP and its analog compounds can produce exactly the same metabolic, neurochemical, and behavioural changes that are seen in schizophrenic patients (Fig. 3). This observation was very important, it emphasized the role of NMDA receptor hypofunction in the development of the disease (29). On the other hand, ketamine showed different effects on healthy individuals, these effects are based on the dose used: at low doses, both psychotic features and cognitive deficits were observed, whereas, anaesthetic effects were present at high doses (31). Post-mortem studies of brain subjects with schizophrenia revealed an increased level of N-acetylaspartylglutamate (NAAG) which is an endogenous NMDA receptor antagonist that binds to glycine site of the receptor in order to prevent NMDA receptor-dependent long-term potentiation (LTP) in hippocampus (LTP: is a kind of synaptic plasticity, where the synapses are being strengthening persistently, it plays important role in memory). They also show decline in the GCPII (a catabolizing enzyme that catabolizes the degradation of NAAG to N-acetylaspartate and glutamate) amount in limbic regions in brain individuals with schizophrenia. All these evidences point to the role of NMDA receptor hypofunction in schizophrenia (32).

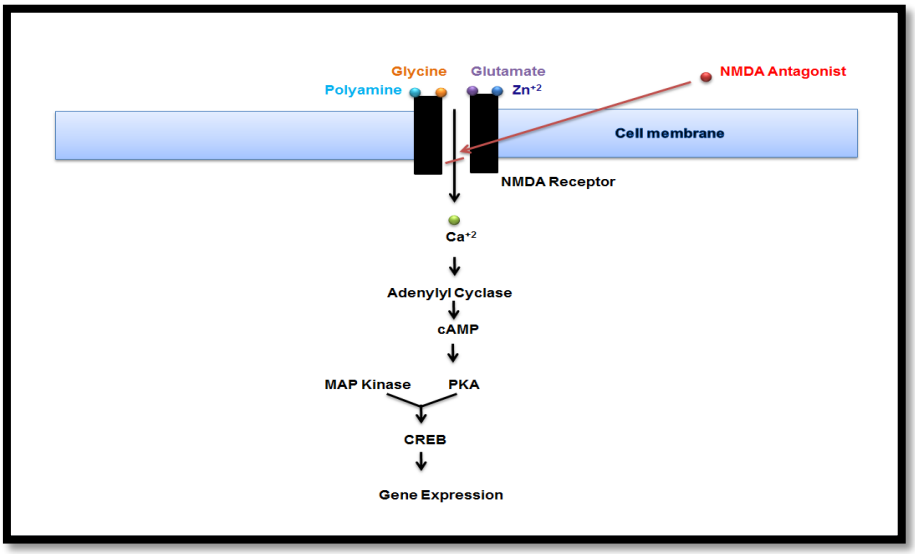

Fig. 3. NMDA antagonist blocks the entry of Ca^{+2} into the cell. The effect of NMDA antagonist on normal receptor transmission.

In the hippocampus, NMDA contributes to the initiation rather than maintenance of LTP. The induction of LTP is prevented by NMDA receptor antagonists, but if these antagonists are added after the induction of LTP, no effect is produced. Consequently, individuals with schizophrenia have a problem with memory formation, not retention since the NMDA mediated part is only affected, while the hippocampus is structurally stable (31). Extensive postmortem and *in vivo* neuroimaging research has described the involvement of the hippocampus in the pathophysiology of schizophrenia. Lieberman and co-workers developed a pathophysiologic model that characterizes the progression of schizophrenia from the premorbid through the prodromal stages to syndromal psychosis. Their longitudinal study of high-risk patients exposed a specific spatiotemporal pattern of hippocampal dysfunction that progresses in the transition from pre-syndromal stages to syndromal psychosis. Their model assumes that, during pre-syndromal stages, dysregulation of glutamate neurotransmission occurring in the CA1 region of the hippocampus which elevates neuronal activity reflected in metabolism and blood flow. As this persists, it drives the transition process to the later prodromal stage and subsequently syndromal psychosis. As the incipient illness progresses, this pathologic process spreads from CA1 to the subiculum and likely beyond the hippocampus and causes an atrophic process in which the neuropil of hippocampal cells is reduced and interneurons are lost. They examined this pathophysiological hypothesis in a rodent model with three experiments. First, they discovered that ketamine-evoked increases in extracellular glutamate in mice mirrored the evoked fMRI pattern, with maximal changes found in the CA1 and subiculum hippocampal subregions. Second, they found that the ketamine-induced effects were associated with atrophy in a spatial-temporally concordant manner. Third, they discovered that the sustained basal hypermetabolism and hippocampal atrophy were decreased or inhibited when the extracellular glutamate efflux was prevented by pretreating with an agent that inhibited ketamine-induced extracellular glutamate efflux, before the intermittent ketamine administration. Therefore, reducing extracellular glutamate is a valid target for preventing or ameliorating the onset of illness and limiting hippocampal atrophy. Thus, It is possible, to design a study in subjects at high risk for psychotic disorders to test whether the glutamate reducing agents i.e. lamotrigine or gabapentin normalize hippocampal hypermetabolism and prevent progression to psychosis and hippocampal atrophy (33).

2.3 Neural Diathesis-Stress Model of Schizophrenia

This model attempts to explain the role of biological (pre-existing vulnerability) and environmental (stress) factors in the etiology and course of the disorder (34). It suggests that people who experience schizophrenia are born with certain genetic or biological predisposition to the disease (Fig. 4), but not all individuals with this genetic or biological susceptibility will develop the illness (35, 36). The diathesis-stress model tends also to explain the relationship between stress and schizophrenia (37). In healthy individuals, stress stimulates cortisol production from HPA axis. The hypothalamus and anterior pituitary stimulate cortisol release from the adrenal glands that lower stress reactivity (Fig. 5). Through a negative feedback loop, high plasma cortisol lowers hypothalamus and pituitary activity (38). In individual with schizophrenia, the homeostasis of the HPA axis is disturbed. This disturbance results in HPA axis hyperactivity and elevated cortisol level that triggers the symptoms of schizophrenia (39).

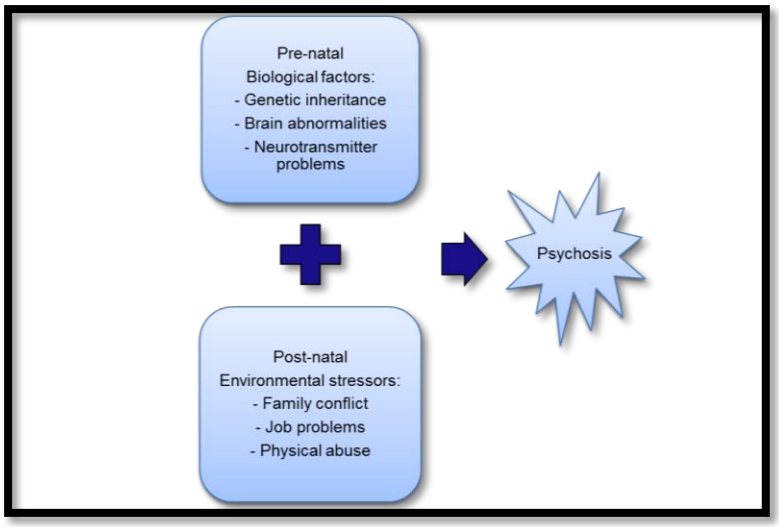

Fig. 4. The neural diathesis stress model. The role of biological and environmental factors in the etiology and course of schizophrenia.

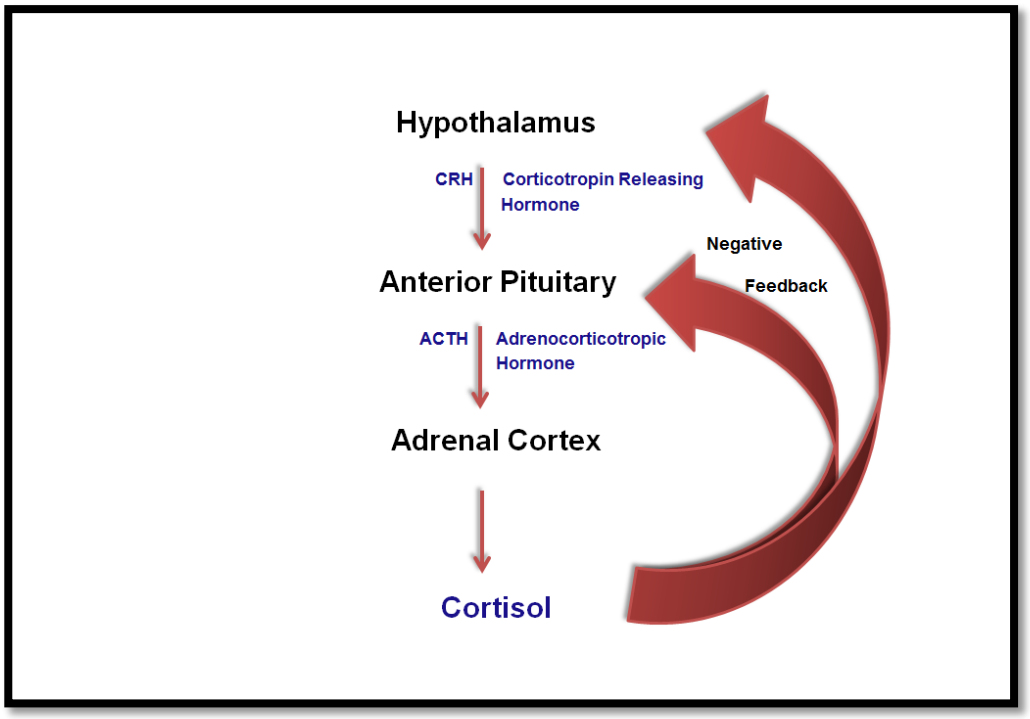

Fig. 5. Regulation of cortisol secretion by HPA axis.

Three chemical messengers are released from HPA axis upon its activation. The binding of these messengers to their corresponding receptors result in secretion of cortisol from adrenal cortex. HPA axis: hypothalamic

pituitary adrenal axis, CRH: corticotropin releasing hormone, ACTH: adrenocorticotropic hormone.

Pituitary volume is reflective of HPA axis structure and function, stress and psychosis severity. Greater perceived distress from adverse life events relates to smaller pituitary volume in people who have a first or second degree relative with schizophrenia. Greater pituitary volume associated with higher nocturnal cortisol in patients with depression or bipolar disorder. In schizophrenia, the pituitary enlarges at the prodromal and early stages and atrophies at the chronic stage. Furthermore, pituitary enlargement is associated with a minimal improvement of psychotic symptoms in early psychosis. Premkumar and colleagues have carried out a study to determine whether cognitive behavioural therapy for psychosis (CBTp) reduces pituitary volume in patients with schizophrenia, and whether pre-therapy memory relates to CBTp-led pituitary volume reduction. They hypothesized that CBTp would reduce pituitary volume and pre-therapy memory would relate to a significant reduction in pituitary volume in patients receiving CBTp. In their study, the pituitary volume was measured at baseline prior to the therapy in 40 patients with schizophrenia and 30 healthy individuals. Later on, pituitary volume was measured again 6–9 months in patients who had received either CBTp with standard care (SC) or SC alone. Both groups were compared based on the change in the pituitary volume from baseline to follow-up. The results showed that the pituitary volume was decreased over time in CBTp + SC patients. In addition, the pre-therapy verbal learning correlated more strongly with the pituitary volume reduction in the CBTp + SC group than the SC group. As hypothesized, pituitary volume reduced over time in the CBTp + SC group relative to the SC group as CBT assists patients to find stress regulation strategies and reduces cortisol level in which lower cortisol relates to lower pituitary volume. In addition, pre-therapy memory related to CBTp-led pituitary volume reduction, such that the association was stronger in the CBTp + SC group than the SC control group, because good memory in patients receiving CBTp could lower stress-related cortisol level (38).

Another research was conducted by Franzen to find the relation between the cortisol release and the severity of symptoms of schizophrenia. He studied this relation in 10 schizophrenic patients. He measured the levels of serum cortisol in these patients while they were on medication, then withdrew the medication and measured the cortisol levels again after 5 weeks. He found increase in the levels of cortisol over the last 5 weeks period which was associated with increase in psychotic episodes (40). This finding was supported by Sachar et al. who carried out similar study on 4 non-medicated patients. They measured the daily cortisol levels in urine over 2-3 month period. They reported that the cortisol levels rose significantly immediately before the psychotic periods. They were higher by 250% compared to the recovery period levels (41).

Numerous animal experiments and human researches proposed that not only cortisol but also DA release are elevated in response to stress (42). Studies have revealed that the glucocorticoids increase the activity of DA in mesolimbic system, while the DA synthesis and receptors increase through activation of HPA. In laboratory animals, the administration of corticosterone augments the rate of DA synthesis in the brain due to the corticosteroids' effects on tyrosine hydroxylase (TH), which is the main enzyme involved in the biosynthesis of catecholamine. Corticosteroids have various effects on TH, they raise not only the enzyme levels but also the rate of transcription of TH gene, the levels of TH messenger RNA, as well as the levels of TH enzyme protein (43). Several studies proposed that activation of HPA axis could alter the DA receptors. They suggested that the prenatal exposure to stress results in no change in D1 receptors, increase in D2 receptors, and declined in D3 receptors in the rats. These effects were not noticed until the animals reached adulthood (35). Antipsychotic treatments reduce dopamine level as well as corticosteroids level. Experiments showed that the longer the duration between onset of schizophrenia

and initiation of the treatment, the worse the course of the disorder and the long-term outcome. The antipsychotic treatment has a protective function, by decreasing the HPA activation that results in the reduction in elevated cortisol level associated with psychosis and inhibiting the response to biological stress which related to psychotic episodes. Therefore, the shorter the non-medicated period of the disease, the less likely of permanent changes in HPA axis; these changes can augment stress sensitivity and symptoms severity (35).

2.4 Neurodevelopmental Hypothesis

This hypothesis was presented in its current formulation by Weinberger, Murray, and Lewis nearly a quarter of century ago (44). The idea that severe mental illness disrupts the normal development of the nervous system had been discussed before, but new evidences have been found that support these findings (45). Firstly, in neuroimaging studies, abnormal brain structure was observed at the onset of the illness, so far no evidence for neurodegeneration was found in post-mortem studies. Secondly, patients with clear illness were suffering from frequent occurrence of cognitive and motor abnormalities at a young age. Lastly, research on primates have demonstrated that the neonatal lesions could have effects later on behaviour, this supports the idea that adult mental illness may have its origin in developmental stages (44, 46).

According to the neurodevelopmental hypothesis, the genetic or environmental factors during crucial early periods of development before the brain approaches its adult anatomical state have adverse impact on the adult mental health (47). It hypothesizes that schizophrenia is the behavioural outcome of an aberration in neurodevelopmental processes that begins long before the onset of clinical symptoms, and that core cognitive deficits are the outcome of an abnormal development of the brain, leading to problems in acquiring cognitive abilities (48-50). The abnormality in brain development and maturation begins prenatally, the brain cells start to generate during the fetal development in the uterus. Neurons build particular pathways in the brain during the second trimester where they start to migrate to their final positions and connect to other neurons (5). This is considered as a critical period in neuronal development, any abnormality in this stage will affect the structural organizations of the brain cells and lead eventually to the development of schizophrenia in young adulthood (Fig. 6) (51). It has been found that early motor disorders and delayed developmental milestones i.e. walking, crabbing and lifting the head are present in those children who later developed schizophrenia (52). This can be seen as early signs of neuropathology and an indication of non-specific neurocognitive abnormalities specific for schizophrenia (53, 54). Walker analysed home videos taken at the early age of people who had later developed schizophrenia and their healthy siblings. Experts who analysed the videos identified those later developing schizophrenia. They found neuro-motor abnormalities especially on the left side of the body and poor motor skills. These deviations were most prominent in the first two years of life where motor skills are developing at the fastest rate (50, 55).

Early studies in experimental neuropsychology and psychopathological research laid the groundwork for subsequent neurocognitive studies. Comprehensive studies proved that cognitive deficits are relatively stable for a long time after onset. These cognitive impairments are present in a milder form in the premorbid and prodromal phase. Moreover, there are some delays in cognitive and neuro-motor skills in those children who later develop schizophrenia. These findings provide solid evidence that there is no neurodegeneration in schizophrenia, and that this disease fits better into what is called a neurodevelopmental disorder. But we still have a limited understanding of what triggers the deviant development in cognitive functioning, and why symptoms do not appear before early adulthood (50).

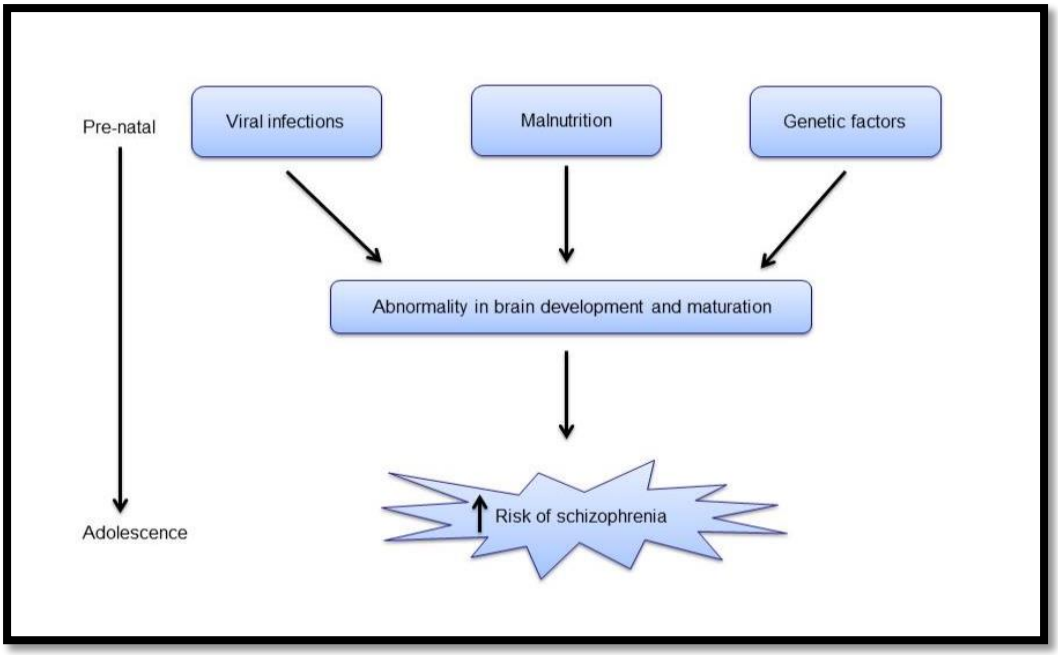

Fig. 6. Neurodevelopmental model of schizophrenia. The adverse impact of genetic or environmental factors on brain maturation during prenatal period and development of schizophrenia.

2.4.1 Risk Factors for Schizophrenia that Impact the Early Brain Development

2.4.1.1 Vitamin D Deficiency

The link between vitamin D deficiency and a wide range of psychiatric illness has become an area of interest for researchers. Prenatal vitamin D deficiency has been suggested as a risk factor for schizophrenia. Evidence from rodent experiments demonstrates that persistent change in the structure and neurochemistry of adult brain results from transient prenatal deficiency in vitamin D (56). In the period between 1988 and 2013, 19 studies were reviewed by one meta-analysis. This review found a strong relationship between schizophrenia and deficiency in vitamin D. In these studies, 2,804 patients with schizophrenia have participated, more than 65% of the patients had deficiency of vitamin D. Individual with vitamin D deficiency are 2.16 times at higher risk of developing schizophrenia compared to individual with sufficient vitamin D (57).

The risk of schizophrenia and status of vitamin D is affected by birth season, high latitude, and skin pigmentation (58). Individuals who are born in winter are more likely to develop schizophrenia later due to reduced exposure to the UV rays that are required for the synthesis of vitamin D. The new-borns in January and February are exposed to lower UV radiation levels during their prenatal and perinatal period. The rate of schizophrenia rises also at high latitudes; this may again be due to lower UV availability and status of vitamin D. At higher latitudes, a comparison between dark-skinned individual and lighter skinned individual shows pronounced reduction in vitamin D in dark-skinned individual, because the individual with lighter skin has less melanin, this allows more effective absorption of UV rays by the skin (Figure 7). It is estimated that at higher latitudes darker skin individuals are more likely to have schizophrenia than the general population (59).

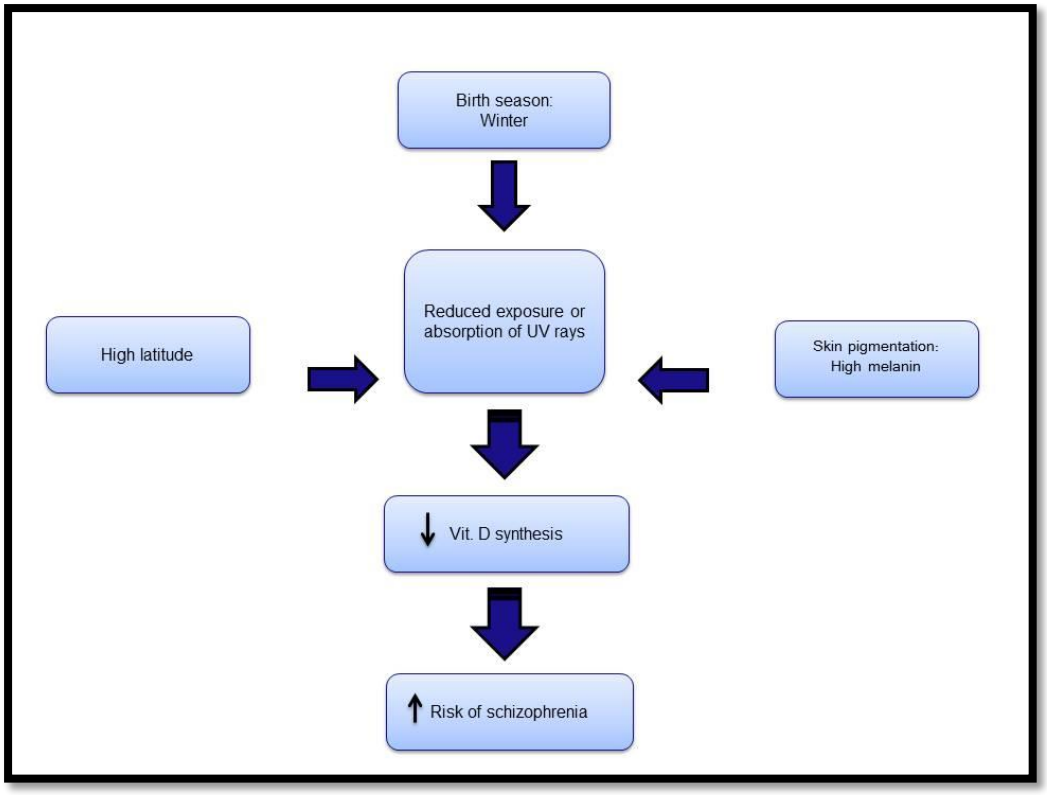

Fig. 7. The link between Vit. D deficiency and risk of schizophrenia. Effect of birth season, high latitude, and skin pigmentation on the status of Vit. D and schizophrenia.

2.4.1.2 Iron Deficiency

Evidence suggests that prenatal nutritional factors may play role in the etiology of schizophrenia (60). During pregnancy, the maternal iron deficiency is the most common nutritional deficiency that adversely affects neurodevelopment which might lead to the development of schizophrenia. Iron is important for the development and maintenance of the functions and structures of the brain, it is essential for myelination and dopaminergic neurotransmission (61). Iron deficiency leads to anemia, where the mother's oxygen-carrying capacity is impaired which results in the reduction of oxygen delivery to the fetus and might cause neurodevelopment disruption through its effect on birth outcomes (62). The risk of iron deficiency depends on the imbalance between iron supply and demand. Research has been conducted on the effect of failure to meet the iron requirements of developing brain on the possibility of developing psychiatric illness. Studies reported that the possibility of developing schizophrenia-spectrum disorder (SSD) in the offspring of low maternal haemoglobin concentration (≤ 10.0 g/dl) is higher by 4 fold compared to the offspring of maternal with mean concentration (≥ 12.0 g/dl). This proposed that the maternal iron deficiency is a risk factor for developing (SSD) among offspring (61).

2.4.1.3 Infection

Over recent decades, evidence demonstrating the relation between prenatal infection and increased risk of schizophrenia has been accumulated (63). The risk of schizophrenia is raised among individuals with prenatal exposure to influenza, rubella, or toxoplasmosis gondi (64). Initially, this evidence was based on ecologic studies,

but recently more studies access the biobanks to examine these hypotheses in analytical settings (56). Individuals who were exposed to infection or immune activation during their fetal life have higher risk of abnormalities in the brain structure and function related to schizophrenia (65). The link between prenatal infections and schizophrenia might be explained by cytokines and chemokines, which are known to mediate the host response to infection. A birth cohort study in northern California concluded that the level of chemokine interleukin-8 in second-trimester pregnant women was 2 fold higher for offspring who developed schizophrenia later compared with controls (66). Yolken and co-workers applied a number of laboratory techniques to examine the effect of infection on the development of schizophrenia. They tested the cerebrospinal fluids (CSFs) that obtained from patients with early stage of schizophrenia. They found increased rate of transcription of HERV-W which is an endogenous retrovirus in the RNA extracted from these fluids. The HERV-W transcription was detected in 30% of the patients with recent onset of schizophrenia and 5% of patients with chronic disease. A number of infectious agents can activate the transcription of endogenous retroviruses. Yolken et al. also examined the presence of active infection in different stages of schizophrenia. The results of their study reported that increased levels of Toxoplasma gondii's antibodies were found in individuals with recent onset of schizophrenia. They also found that serological evidence of infection with Toxoplasma gondii and Herpes Simplex Virus type 1are related to increased levels of cognitive impairments in patients with early stage schizophrenia (67).

In childhood, the CNS viral infections have been linked to increased risk of adult psychotic illness via two mechanisms, direct effects of pathogenic microorganism, and the effects of inflammatory response on the brain (68). Inflammation leads to release of peripheral inflammatory cytokines, including IL-6 or tumor necrosis factor alpha (TNF-α), these cytokines communicate with the brain via three different ways: vagus nerve, active transport, or enter through a leaky circumventricular area in blood-brain barrier. When these inflammatory cytokines reach the brain, their signals stimulate the microglia to secrete local inflammatory mediators (cytokines, chemokines, and proteases) from their monocyte and macrophage lineage. These local inflammatory mediators have several effects: First, they affect the function of the neurons and synaptic plasticity, second, alter the metabolism and reuptake of neurotransmitters i.e. dopamine and serotonin. Finally, they stimulate the HPA axis (69). In older adults, the cognitive and functional decline after systematic infection might be associated with a systematic proinflammatory response in the brain (70). The activation of peripheral immunity in healthy volunteers showed raised levels of circulating cytokines, induced anxiety, low mood, and declined cognitive performance (65).

2.4.1.4 Advancing Paternal Age

Advanced paternal age has been linked to a range of neurodevelopmental disorders including autism and schizophrenia (56). During early childhood, the offspring of older fathers have impaired neurocognitive development. A meta-analysis demonstrated that the risk of schizophrenia increased in the offspring of older fathers (aged $\geq$ 30 years) compared with younger fathers (aged $\leq$ 29 years) (71). The greatest risk was found in fathers aged $\geq$ 50 years. Moreover, it was reported that schizophrenic patient without family history of schizophrenia is more likely to have older father than schizophrenic patient with family history of the disease. Therefore, advanced paternal age is argued to be critical risk factor for schizophrenia (72). Wu and colleagues studied the relation between advanced paternal age and schizophrenia. Their research was conducted in a Chinese Han population where 351 schizophrenic patients and 238 healthy volunteers were participated in their study. They investigated the effect of age and sex on the risk for development the illness in the offspring. The result of the study reported an association between advanced paternal age and increased risk for

schizophrenia, and the risk for the illness in offspring both grew in synchrony with advanced paternal age. They found that the higher risk of schizophrenia is associated with later beginning of fatherhood, where the risk of the disease rose from 2.660 to 10.183 in the paternal age range of 30-34 and ≥ 35. On the other hand, they found no association between maternal age at birth and increased risk for schizophrenia in offspring. They also found that there is no significant difference in the effect of advanced paternal age and risk of development schizophrenia in the male and female offspring (73). This finding was supported by another population based cohort study that conducted by Sipos et al. where they also reported no difference in the effect of advanced paternal age on the risk of schizophrenia for both male and female offspring (74). However, the mechanisms behind these linkages remain unclear, but several hypothesised mechanisms suggest that there is a causal relation, whereas others argued that the linkage can be explained by unmeasured disturbing (75).

3. CONCLUSION

This review presents a summary of the available hypotheses and theories that are related to schizophrenia. Each hypothesis proposes a different mechanism that might involved in the development of the illness. The dopamine hypothesis suggests that the schizophrenia develops due to dopamine abnormality in basal ganglia. This theory was supported by the fact that so far D2 receptor antagonists are considered the best available treatment for schizophrenia. As for NMDA hypo activity hypothesis, it has been argued that NMDA hypofunction involves in the pathophysiology of the disease due to the fact that NMDA receptor antagonists produce schizophrenia-like symptoms in healthy individuals. In addition, an increased level of NAAG and decreased level of GCPII were found in brain individuals with schizophrenia. These two theories provided insufficient facts about the etiology of the disease, which led to a conclusion that it is most likely a combination of both theories might explain the etiology of schizophrenia since both neurotransmitters have influence on each other. Later on, a new theory was developed which pointed to the role of genetic and environmental factors in the pathophysiology of schizophrenia. This hypothesis was known as a neural diathesis-stress model of schizophrenia. However, evidences form genetic studies indicated that the adult mental illness may have its origin in development. As a consequence, additionally, to neurotransmitter dysfunction, receptor hypofunction, and environmental factors, other causes may play a role in the etiology and course of schizophrenia i.e. vitamin D and iron deficiencies, infection, paternal age and etc. All these factors are required to be investigated in order to provide a new hope for the people suffering from the illness. Numerous studies are in progress to find the exact pathophysiology of schizophrenia, but despite such progress, there are still many questions that need to be answered. Why it takes more than two decades for the symptoms to be developed? Why the disease not noticed until the patient reach adulthood? What are the exact changes that happened in the brain? All these questions and more require to be answered in order to help us in developing the appropriate therapy for treating the disease.

Conflict of interest: The authors declare that there is no conflict of interest.

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الفصام: آلية المرض المبهمة

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ملخص

الخلفية: يعد مرض الفصام واحدا من أشد الاضطرابات التي تعيق حياة الأشخاص المصابين بالمرض. على الرغم من معدل انتشاره المنخفض، إلا انه يؤثر سلبًا على حياة المرضى، عائلاتهم والمجتمع. تعالج مضادات الذهان المتوفرة حاليا الأعراض الإيجابية فقط، ولكنها لا تحسن الأعراض السلبية أو المعرفية لمرض الفصام. لذلك، يتم إجراء أبحاث مكثفة من أجل اكتشاف مركبات داوئية جديدة لمعالجة مرض الفصام والسيطرة على أعراضه لكن لن يتحقق ذلك إلا من خلال الفهم الكامل لآلية المرض المبهمة.

الطرق: لقد تمت مناقشة أربعة فرضيات تشرح الآليات المحتملة التي قد تكون متضمنة في تطور مرض الفصام في هذه المقالة حيث تمت الإشارة إلى تأثير نقص فيتامين دال والحديد، الإصابة بالالتهابات، بالإضافة الى عمر الآباء على احتمالية إصابة الأبناء بمرض الفصام، وقد تم أيضا مناقشة بعض الدراسات السريرية ونتائجها.

النتائج: لا يزال السبب الرئيسي للإصابة بمرض الفصام غير معروف بشكل دقيق. حيث تشير بعض الدراسات ان المرض قد يحدث نتيجة لخلل في الناقل العصبي، أو نقص في الأداء الوظيفي للمستقبلات، أو العوامل البيئية، أو عوامل أخرى قد تلعب دورًا في ظهور هذا المرض وتطوره.

الخاتمة: كل العوامل التي تم مناقشتها في هذه المقالة قد تكون سببا في حدوث مرض الفصام، مع ذلك يجب التحقق من دور هذه العوامل في ظهور المرض من أجل توفير أمل جديد للأشخاص الذين يعانون من مرض الفصام، لذلك فان العديد من الدراسات الحالية تهدف لمعرفة الفيزيولوجيا المرضية الدقيقة للفصام، ولكن على الرغم من هذا التقدم، لا تزال هناك العديد الأسئلة التي يجب الإجابة عليها لتساعدنا في تطوير العلاج المناسب لهذا المرض.

الكلمات الدالة: مضادات الذهان، الإعاقة، الفرضيات، الآليات، الفصام.

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Molecular modeling studies of some phytoligands from *Ficus sycomorus* fraction as potential inhibitors of cytochrome *CYP6P3* enzyme of *Anopheles coluzzii*

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ABSTRACT

The major obstacle in controlling malaria is the mosquito's resistance to insecticides, including pyrethroids. The resistance is mainly due to the over-expression of detoxification enzymes such as cytochromes. Insecticides tolerance can be reduced by inhibitors of P450s involved in insecticide detoxification. Here, to design potential CYP6P3 inhibitors, a homology model of the enzyme was constructed using the crystal structure of retinoic acid-bound cyanobacterial CYP120A1 (PDB ID: 2VE3; Resolution: 2.1 Å). Molecular docking study and computational modeling were employed to determine the inhibitory potentials of some phytoligands isolated from *Ficus sycomorus* against *Anopheles coluzzii* modeled P450 isoforms, *CYP6P3*, implicated in resistance. Potential ligand optimization (LE) properties were analyzed using standard mathematical models. Compounds 5, 8,and 9 bound to the Heme iron of *CYP6P3* within 3.14, 2.47 and 2.59 Å, respectively. Their respective binding energies were estimated to be -8.93, -10.44, and -12.56 Kcal/mol. To examine the stability of their binding mode, the resulting docking complexes of these compounds with *CYP6P3* were subjected to 50 ns MD simulation. The compounds remained bound to the enzyme and Fe (Heme):O (Ligand) distance appeared to be maintained over time. The coordination of a strong ligand to the heme iron shifts the iron from the high- to the stable low-spin form and prevented oxygen from binding to the heme thereby inhibiting the catalytic activity. The LE index showed the high potential of these compounds (5 and 8) to provide a core fragment for optimization into potent P450 inhibitors.

Keywords: Homology modeling; CYP6P3; Molecular docking; Molecular dynamics simulation ligand efficiency; CYP6P3 inhibitors.

INTRODUCTION

Malaria vector control strategy generally uses traditional approach to decrease global malaria cases. These measures include space spraying, indoor residual spraying, long-lasting insecticidal nets, larval source management, bio-larvicide application, etc [(1)]. The world health organization (WHO) recommends the use of pyrethroid insecticides only for adult mosquitoes in current vector control program [(1)(2)]. In recent times, resistance of the adult mosquitoes to these insecticides was reported widely, making research targeted at new classes of insecticides incredibly important [(1)(3)]. Also, some insecticides/pesticides have apparent environmental shortcomings, due to their persistence in the surroundings. These persistent insecticides are prone to resistance by

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insects much more commonly than non-persistent insecticides. Photo-degradation by sunlight is one of the pathways that destroy pesticides when release to the environment [4].

Cytochrome (P450) monooxygenase, a protein of 45–55-kDa, is a structurally different family of hydrophobic, heme-containing, membrane associated enzymes involved in the metabolism of xenobiotics such as insecticides in mosquitoes, thereby causing resistance. In insects, P450s are inducible and metabolize diverse collection of substrates. This usually results in the detoxification of the insecticide to more soluble and less toxic forms. Thus, P450s play important role in numerous aspects of insect biology, physiology and the insecticide resistance [5][6]. The resistance linked to P450s occurs probably due to gene(s) over expression [6]. Cis- and trans-regulating factors involved in P450 gene expression are also implicated in the insecticide resistance. This may be responsible for the resistance and consequences of malaria cases in the region. The mosquitoes have a genomic collection of over 100 P450 genes [7][8][9], that can mount defense against the insecticides leading to insecticide resistance. The *CYP6* family of P450 enzymes has been reported to be up-regulated in the majority insecticide resistant malarial vectors [10]. *CYP6M2*, *CYP325A3*, **CYP6Z1**, **CYP6Z2** *CYP6Z3*, *CYP6P3*, **CYP9K1** and *CYP4G16* [10][11][12][13], has been established to be up-regulated in adults pyrethyroids resistant mosquito strains. These enzymes are recognized for mono-oxygenase activities, in which they catalyze the transfer of one atom of molecular oxygen (O_2) to a substrate (e.g., insecticides) and reducing the other oxygen to water during the reaction. They also show other catalytic properties such as being oxidases, reductases, desaturases, dealkyalases, isomerases, C-C cleavage and dimerization reactions [14].

P450s almost always act as monooxygenases, or mixed-function oxidases, using the stoichiometric mechanism shown in equation (1).The reaction utilizes the pyridine nucleotide NADH or NADPH as a cofactor which deliver electrons via a flavoprotein or an iron-sulfur protein. [15]

$$\mathbf{R + NAD(P)H + O_2 \longrightarrow NAD(P)^+ + RO + H_2O} \quad \ldots\ldots(1)$$

Where: R is the insecticide or xenobiotics.

The P450s enzymes are generally inhibited by mechanism-based inhibition through covalent modifications of the active site amino acid residues and / or the central heme group of the enzyme which result to irreversible enzyme inhibition [16]. This P450s inhibition leads to prevention of insecticides detoxification in mosquitoes and restore the efficacy of the insecticides in mosquitoes' resistance strains. The most frequently use P450s inhibitor nowadays is Piperonyl butoxide (PBO). It has being use as insecticide synergist of pyrethroids against mosquito vectors [17][18]. Additive or synergistic applications through combination of PBO and another insecticide such as pyrethroids with different mode of action may completely restore the efficacy of pyrethroid insecticides on resistant *Anopheles* mosquitoes [19][20].The PBO have reported to have some environmental, non-target organisms and human toxicities [21][22]. As such, it warrants formulating a new insecticides or synergists or potentiating agents that are human and eco-friendly [20].

Over-expression of *CYP6P3* isoform of P450 has been reported in Benin and Nigeria malaria vectors [23]. The *CYP6P3* isoform is known to metabolize insecticide such as pyrethroids and bandiocarb [24][25]. After many decades of insecticides pressure, mosquito populations have become resistant to multiple chemical insecticide families, compromising the effectiveness of chemical-based control [26]. Many insecticides, including the organochlorines, organophosphates and carbamates, persist in the environment as toxic waste [27] and are neurotoxic not only to humans, but to livestock [28]. Furthermore, the redundant mode of action of insecticides may accelerate the emergence of cross-resistance to other insecticides [27].

The resistance of insects to insecticides can be minimized or enhanced by inhibitors of cytochrome P450s

involved in insecticide detoxification. Today, there is an urgency to develop alternative control methods, including novel insecticides, to better manage resistance and maintain effective tools for fighting vector-borne diseases such as malaria. Insights into molecular mechanisms of interactions of natural compounds with some mosquito P450s implicated in resistance can be of important for the rational design of new insecticides or insecticide synergists, and for insecticide resistance management control of malaria vectors. The insecticidal and acricidal activities of plant called *Ficus sycomorus* have been reported by Rhome [(29)]. The study identified 22 main compounds in the leaf extract that were more toxic in fumigant toxicity test than contact phase to insects. *Ficus benghalensis* and *Ficus sarmentosa var. henryi* were proven to have larvicidal activities against different larval stages of both *Culex* and *Anopheles* mosquitoes [(30)].

Phytochemicals act at multiple, novel target sites [(31)(32)(33)(34)], thereby reducing the potential for resistance [(32)(35)(36)]. Some bioactive compounds, rhinacanthin-A, -B, and –C (Figure 1), isolated from *Rhinacanthus nasutus* exhibited potent inhibitory activity against both CYP6AA3 and CYP6P7 isoform from *Spodoptera frugiperda* cells [(37)].

Natural insecticides such as azadirachtin, pyrethrins, rotenone, spinosad and abamectin were proved to be effective against insects [(38)]. But selectivity durability and safety of botanical insecticides are not total and some natural insecticidal compounds are very toxic to humans and non target organisms [(38)]. Pyrethrum or pyrethrins, extracted from *Chrysanthemum cineraria* seed has been used locally as an insecticide to control insects for centuries. The pyrethrins are particularly labile when exposed to the UV element of sunlight (Fig. 1). This fact significantly restricted the use of this natural insecticide outdoors. A study previously reported the pyrethrins half-life on tomato and bell pepper fruits grown in a field were 2 h or less than that [(39)]. This problem of pyrethrins labiality created the need for the search of new or development of synthetic derivatives that are more stable in sunlight, effective, non-toxic to non-target organisms and eco-friendly.

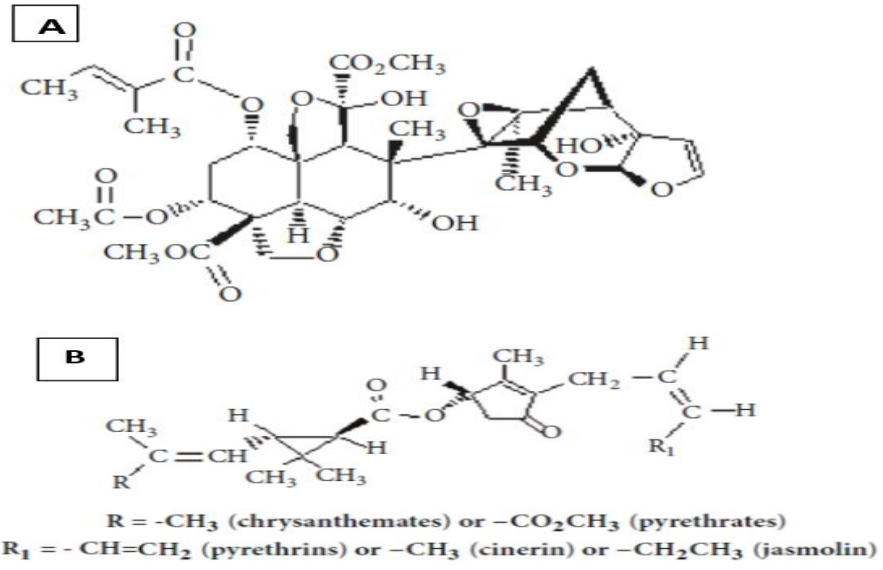

Figure 1. Chemical structures of some natural insecticides: Azadirachtin (B) and Pyrethrins and its derivatives (B) [(38)].

According to Jones et al.[40], understanding the complexity of insecticide resistance mediated by P450s relies on homology modeling since the crystal structures is not available. Such modeling, as explained by de Graaf *et al*. [41], has greatly improved the understanding of the complexity of insecticide resistance. In this study, homology model of *CYP6P3* is built and binding of *Ficus sycomorus* phytoligands to the protein was investigated using molecular docking. Molecular docking allows for prediction of binding mode and binding affinity of these potential inhibitors. Ligand efficiency (LE) calculation was performed to further evaluate the potency of the compounds. Molecular dynamics simulation was used to rapidly validate results obtained from both docking and LE calculation.

Methods

Computational Methods

Homology modeling and system setting

FASTA sequence files of the target proteins were retrieved from the Uniprot database [42]. Crystal structure of retinoic acid bound cyanobacterial CYP120 A1 (PDB ID: 2VE3; Resolution: 2.1 Å) was retrieved from the protein data bank [43]. Modeler structure prediction software [44] was used to construct the homology model of mosquito's *CYP6P3*. Structural validation was carried out using ProSAweb server (https://prosa. services. came. sbg. ac.at/prosa.php) on which the overall quality of the model was calculated by comparing its z-score with the z-score values of the protein structures determined experimentally. This model was found to be within the region of structures determined by X-ray crystallography (Figure 2A), and with minimum residual energy content (Figure 2B). The model was then prepared, preprocessed, corrected the bond orders, added hydrogens and disulfide bonds where necessary, assign correct atom charges based on the protonation state using predicted pKa values at physiological pH. The charge state was optimized, and restrained minimization was carried out.

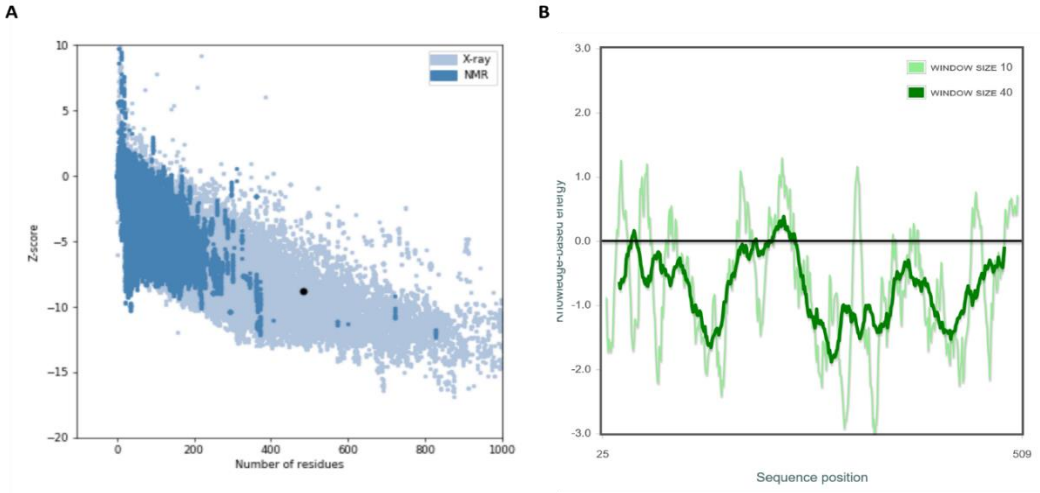

Figure 2. Structural validation of the best model: ProSA-web z-scores of protein chains from protein data bank that were determined by X-ray crystallography (light blue) and NMR spectroscopy (dark blue) with respect to their length. The plot showed that CYP6P3 (black spot) with the z-score value -8.00 is within the range of native conformation (A). The energy plot showing the local model quality by plotting energies as a function of amino acid sequence position i—positive values correspond to erroneous parts of the model (B).

Molecular docking and ligand optimization calculation

Dockings were done using the Glide software [45]. The extra precision (XP) scoring function of the GLIDE was used to model ligand-protein interaction with increased accuracy [46][47]. The template crystal structures were used to create docking grid file. The grid file was generated using a van der Waals scaling factor of 1 and a partial charge cutoff of 0.25. Ligands were docked into the generated grid of the protein receptor using an OPLS3 force field and their docking scores were calculated using Glide XP scoring function [48].

The XPG score optimized the ligand binding energy on the behalf of the force field parameters, and penalties that had significant influences over the receptor-ligand binding. The following equation denotes the formulae for XPG calculations.

Score= a*vdW+b*Coul+Lipo+Hbond+Metal+BuryP+RotB+Site…….(2)

Where: vdW, Coul, Lipo, H bond, metal, BuryP, Rot B, and Site denote van der Waals energy, Coulomb energy, lipophilic contacts, hydrogen-bonding, metal-binding, penalty for buried polar groups, penalty for freezing the rotatable bonds, and polar interactions with the residues in the active site, respectively; a = 0.065 and b = 0.130 are coefficient constants of van der Waals energy and Coulomb energy, respectively.

Ligand optimization predictions

The Ligands optimization parameters were determined using mathematical equations below:

$Ki = 10(\Delta G/1.366)$ [49]…… .(3)

$LE = \Delta G° / HA = (-2.303RT/HA) \times \log(Kd/C°)$ (LE ≥0.3)[50]…… ……..(4)

Where: $\Delta Go = -2.303RT \times \log (Kd/C°)$

R is the ideal gas constant ($1.987x10^{-3}$ kcal/K/mol) and **T** is the temperature in Kelvin (K), Co is the standard concentration, and Kd is the binding constant.

The above formulae assumed that the binding ΔG is directly proportional to the number of heavy atoms or non-hydrogen atoms in the ligand. And the standard reference assumption for the formulae is aqueous solution at 300 K, pH = 7, all other concentrations being 1 M. At this experimental state, the formulae term -2.303RT is approximately -1.37 kcal/mol, when the Kd is expressed as the logarithm to base 10 (log Kd). LE does not state that a change in the heavy atom count of +1 results in a log order change in affinity (pKd=1) [51]. Projected suitable values of LE for good candidates are >~0.3 kcal/mol/non-hydrogen atom (HA) (based on a <10nM molecule having HA of 38 (~500 Da) [52] and cLogP (Desolvation) of <3 [53].

To play down the important of ligand size in the binding affinity, two size-independent modifications of LE indices using heavy atom count only have been proposed. These are Fit Quality (FQ) [54] and Size Independent Ligand Efficiency (SILE) [55].

Fit Quality (FQ) = LE/LE_{scale}……(5)

$LE_{scale} = 0.873e^{-0.026 \times HA} - (0.064)$ (FQ ≥0.8 as hit) [52]……..(6)

SILE = pIC50 or pKi ÷ $HA^{0.3}$ ……(7)

Or = $-RT\ln (pKi)/ (NHA)^{0.3}$……(8)

Reducing the ligand size and lipophilicity tends to increase ligand efficiencies, and the individual target datum suggests that doing so may not necessarily be detrimental to affinity for many targets.

Molecular dynamics simulation

The free form of *CYP6P3* enzyme and its docking complexes with compounds 5, 8, and 9 were prepared for MD simulation. Input files were generated using CHARMM-GUI server (http://www.charmm.org) [56], via which the ligands were parameterized using CHARMM General Force Field (CGenFF) server (https://cgenff.paramchem.org/) [57]. MD simulation was performed using Nanoscale MD (NAMD) software [58]. A 1000-steps minimization by steepest descent method; 5 ns equilibration in standard number of particles, volume, and temperature (NVT) ensemble; and unrestrained 50 ns-

production MD simulations in standard number of particles, pressure, and temperature (NPT) ensemble were performed. The simulation was carried out at 2 fs time scale, and the trajectory frame was collected every 100 ps. To identify the dominant structure, each trajectory was clustered using RMSD cutoff of 3.0 Å using Chimera[59]. Ligand binding mode stability was assessed by computing root mean-square deviation (RMSD), root mean-square fluctuation (RMSF), radius of gyration (Rg), and Fe (Heme)- O(Ligand) distance over the entire simulation period.

Results

Molecular docking of phytoligands in the active site of *CYP6P3* is presented in table 1 below. Eight (8) compounds from the GC-MS analysis of active fraction of *F. sycomorus* with 1 standard inhibitor (Compound 9- the PB0) (Fig. 3) were docked into the active site of the enzyme. The binding energies for compound 1, 2, 5, 8 and 9 were 3.77, 2.89, 8.93, 10.44 and 12.56 Kcal/mol, respectively. However, compounds 3, 4, 6 and 7 were too large to fit into the active site of the *CYP6P3*. Compounds 5, 8 and 9 bound to the heme-iron at a regioselective distance or putative hydroxylation sites of 3.14 Å (Fig. 4), 2.47 Å (Fig. 5) and 2.59 Å (Fig. 6), respectively, which is less than the maximum distance required for reactivity (6 Å) with Fe-atom of the heme group. The common interacting amino acid residues in the binding pocket were *Phe123*, *Val310*, *Pro379* and *Val380* (Fig. 4-6). These and other residues contributed to the stability of the enzyme-ligand interactions via hydrophobic and π-π interactions (Fig. 4-6). Estimating the binding affinity of a small molecule in the active site of the receptor helps to understand ligand-protein interaction. These molecular interactions occur through hydrogen bond and phi-interaction. Phyto-ligands 5and 8 bound well to the heme iron at close distance to *CYP6P3* and thus may be potential inhibitors (Table 1). The amino acid residues such as *Phe123* close to the heme/porphyrin of the modeled *CYP6P3* indicates that it may be involve in π-π interactions with the heme ring, and make available, the electrons that can be supplied for the formation of the activated and stabilizing oxygen atom during the reaction with the ligand (Fig. 4-6). Other amino acid residues lining the active site have no π-electron to contribute except for the hydrophobic side chains (Fig. 4-6). These side chains could be engaged in steric interaction with the heme group (Fig. 4-6).

Table 1: Binding energies and Ligand-heme iron Distance of *CYP6P3* interactions with *F. sycomorus* phytoligands

Compounds	Name of Ligand	ΔG (Kcal/mol)	Distance from heme (Å)
1	Dodecane, 4,6-dimethyl-	-3.77	-
2	Heptadecane	-2.89	-
3	Eicosane	Non-inhibitor	-
4	4,8,12,16-Tetramethylheptadecan-4-olide	Non-inhibitor	-
5	Bis(2-ethylhexyl) phthalate	-8.93	3.14
6	Tetracontane	Non-inhibitor	-
7	Squalene	Non-inhibitor	-
8	Sigmasterol	-10.44	2.47
9	Piperonyl butoxide (PBO)	-12.56	2.59

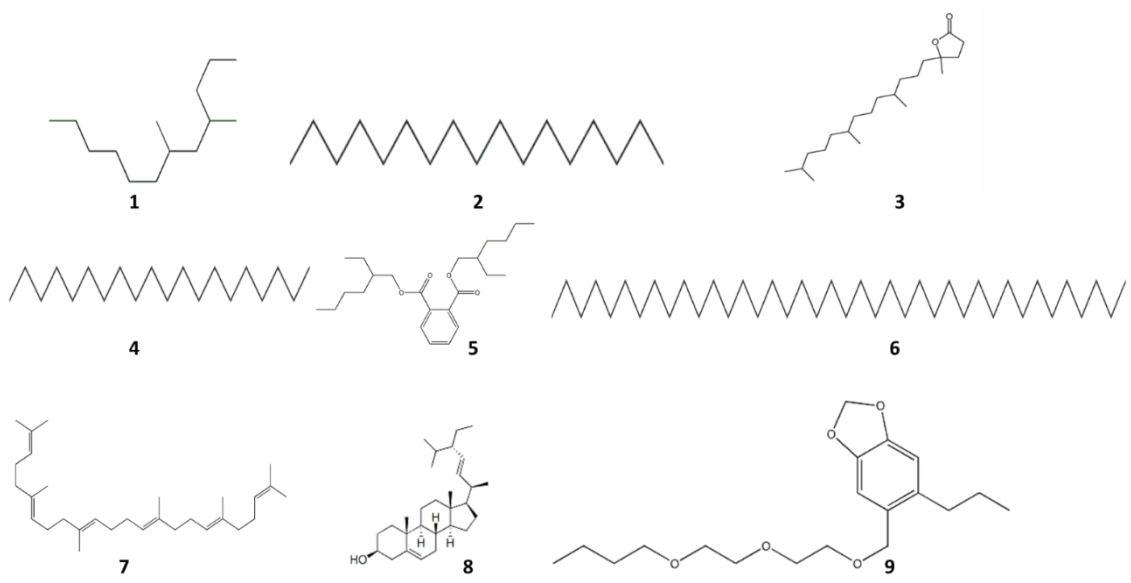

Figure 3. The phytoligands from active fraction of *Ficus sycomorus* obtained from GC-MS analysis.

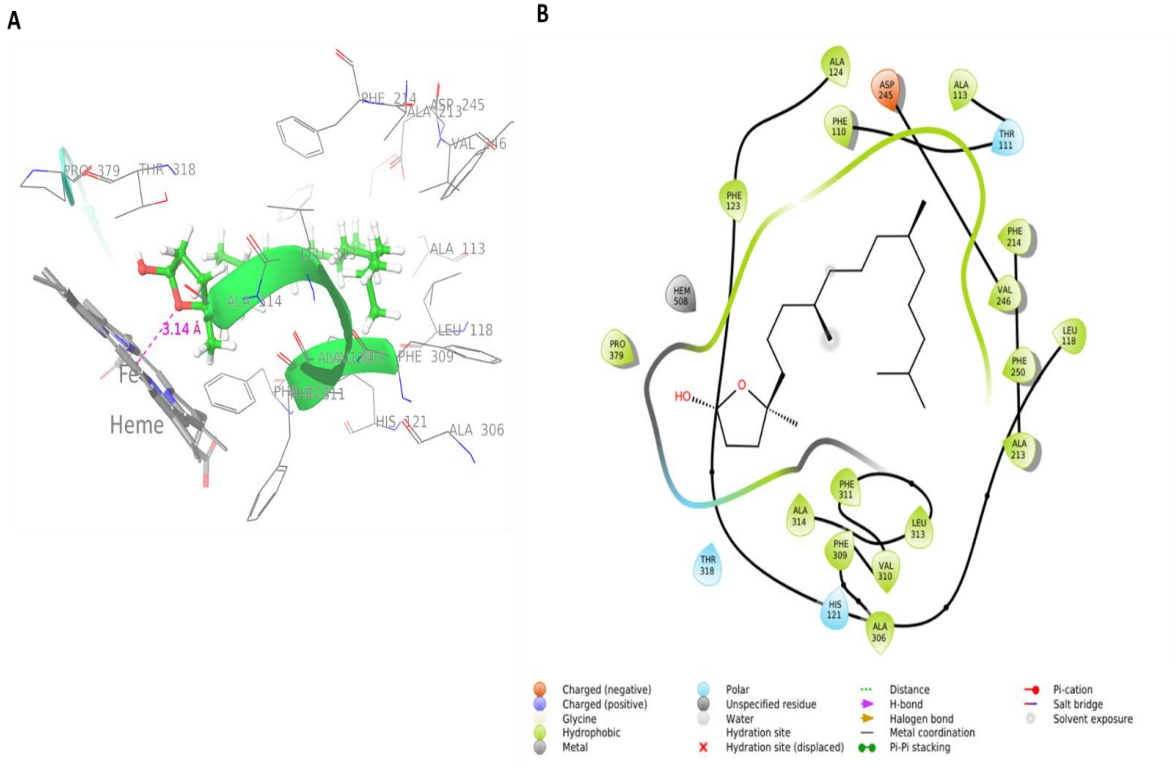

Figure 4. Docking pose and interaction diagram of compounds 5 with *CYP6P3*. Compound 5 bound to *CYP6P3* by coordinating with Fe^{2+} of the heme group via oxygen and hydroxyl groups.

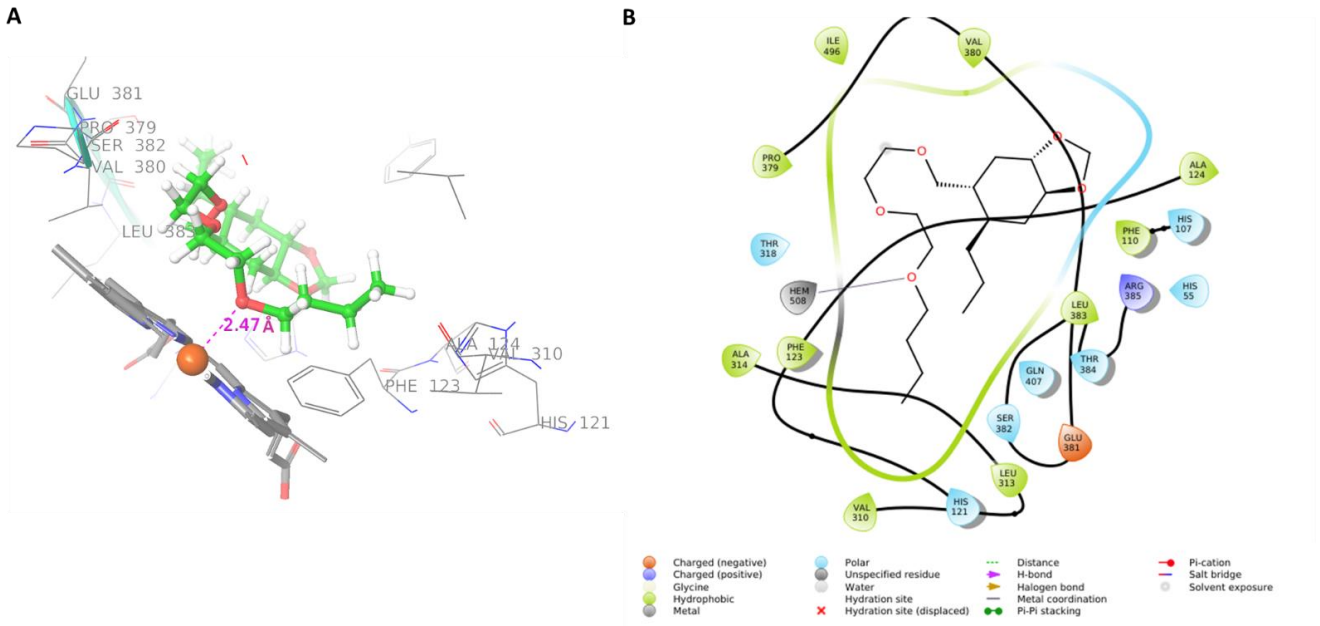

Figure 5. Docking pose and interaction diagram of compounds 8. Compound 8 bound to *CYP6P3* by coordinating with Fe^{2+} of the heme group via oxygen and hydroxyl groups.

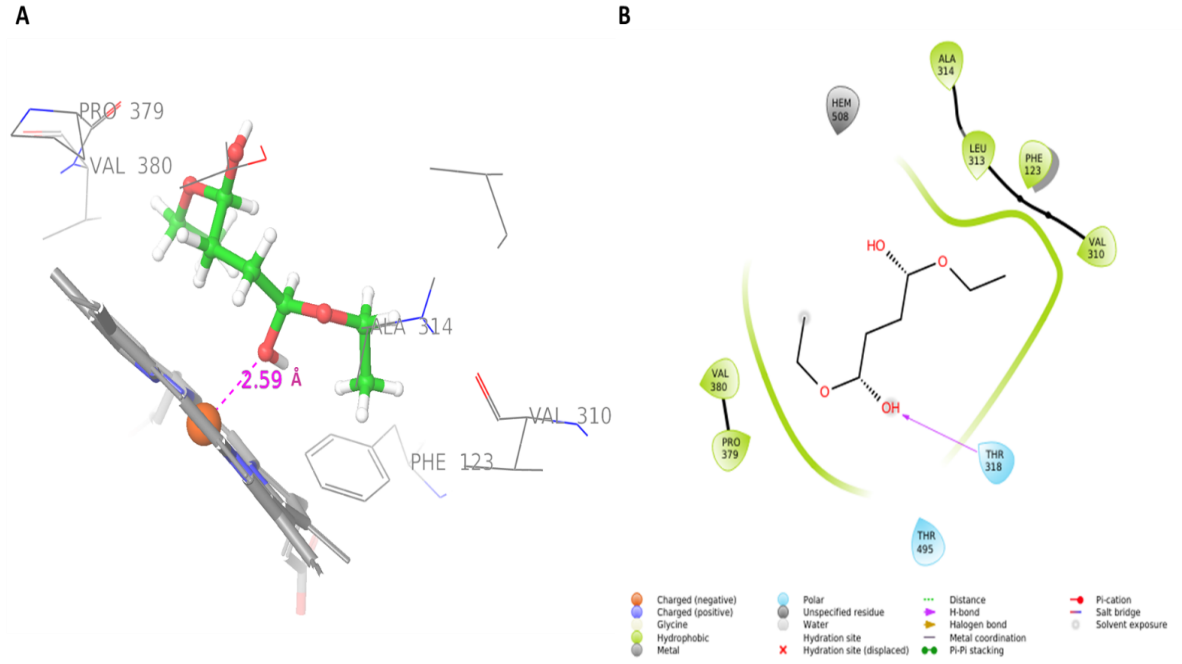

Figure 6. Docking pose and interaction diagram of compounds 9. Compound 9 bound to *CYP6P3* by coordinating with Fe^{2+} of the heme group via oxygen and hydroxyl groups.

Ligand optimization prediction

From molecular docking studies, most of the pytoligands appeared to be too big to fit into the active site of the enzymes generally due to molecular structure and size. The ligand optimization potentials of these promising fragments were evaluated, and the result is presented in Table 2. The ligand efficiency parameters: ligand efficiency, size independent ligand efficiency and Fit quality potentials of the fragments from *F. sycomorus* active fraction (LE, SILE and FQ) showed characteristics potential for molecular optimization.

Table 2: Physiochemical/bioactivity prediction of Phytoligands from *F. sycomorus* for potential optimization

Compound	Ki(M)	LE(kJ/mol/HA	SILE	LEscale	FQ
1	27.60	0.27	12.49	0.54	3.65
2	21.16	0.17	9.04	0.50	2.48
5	65.37	0.37	24.03	0.36	6.47
8	76.43	0.36	27.59	0.34	7.50
9	-	-	-	-	-

Compounds 5 and 8 have LE of 0.37 and HA of 0.36 kcal/mol/, respectively. This is within the range of standard LE of developable drug fragment (≥ 0.3Kcal/mol/HA). While compound 1 can be enhanced, compound 2 appears to be poor target fragment with LE 0.17 kcal/mol/HA (Table 2). The SILE and FQ of these phytoligands were good with the best being compound 8 (FQ= 7.50; SILE = 27.59) and can be good starting material for optimizations.

Molecular dynamics simulation

Molecular dynamics simulation has proven to be a powerful tool used for examining the stability of ligand binding mode [(60)] The four systems simulated showed increased RMSD trend until halfway through the simulation, beyond which convergence was reached until the end of the simulation (Fig. 7A). This increased RMSD trend of the modeled structure of *CYP6P3* showed structural adjustment and transition to potential active structure [(61)]. Nevertheless, the ligands slightly reduced the structural deviation toward the end of the simulation. These trends are consistent with residual fluctuation (RMSF) (Fig. 7B), and radius of gyration—a measure of protein structural compactness (Fig. 7C). Furthermore, owing to the potential role of Fe^{2+}coordination by ligand, timeline of distance between Fe^{2+} and O on the ligands was computed. This distance showed stable trends (especially, for compound 8 and 9) over time (Fig. 7D).

Trajectory clustering identified dominant structure representing 78%, 69% and 64% respectively, compound 5, 8, and 9 systems. For these structures, the Fe^{2+}:O distance was found to be 2.98 (Fig. 8A), 2.77 (Fig. 8B) and 2.27 Å (Fig. 8C) respectively, for compound 5, 8, and 9.

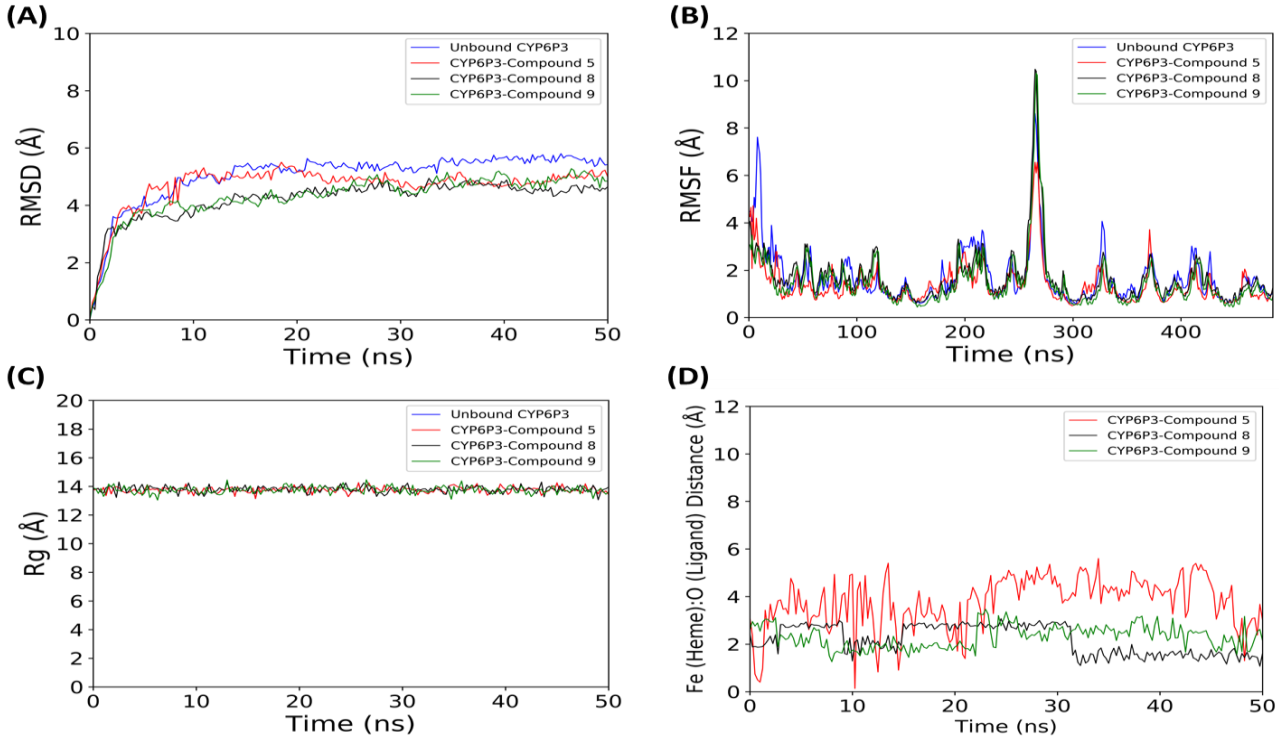

Figure 7. Molecular dynamics simulation results: (A) root mean-square deviation (RMSD) (B); root mean-square fluctuation (RMSF); (C) radius of gyration; and (D) Fe^{2+}:O distance profiles.

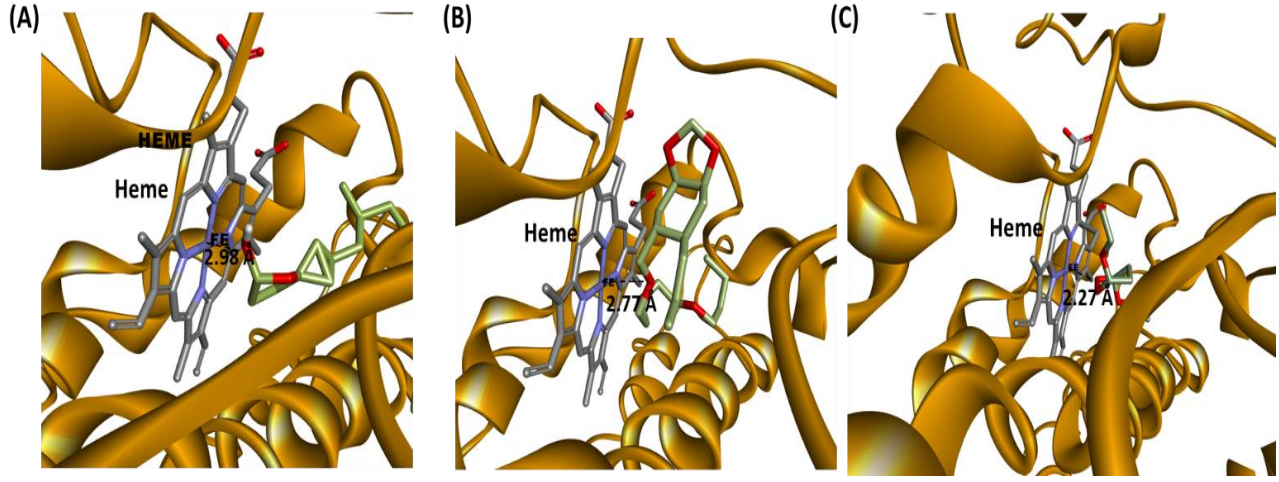

Figure 8. Analysis of binding mode of ligands following 50 ns MD simulation: (A) compound 5; (B) compound 8; and (C) compound 9.

Discussion

Computational studies using molecular docking and modelling are generally vital tools used by many researchers for new drug design and development. The docking simulations are also reported as a useful tool, illustrating for example, the cytochrome P450 isoforms that can metabolize insecticides. The docking simulation can also rationalize the observed heterogeneities of substrate preferences and metabolism detected among the P450 enzymes family [(62)(63)]. In the absence of crystal structure of P450 isoform, the knowledge of the molecular structure of the insect P450s have only been possible through homology modelling [(40)]. *In silico* homology modelling study is an explanatory tool rather than predictive tool [(64)], and can be used to investigate structure-function of the enzyme targets.

The docking scores showed promising inhibitory potentials of some pytoligand from *F. sycomorus* active fraction against *CYP6P3* isoform. Out of the 10 compounds assessed, only 4 ligands fitted into active site of the enzyme— 3 of which formed contacts with the central heme iron, whereas the rest were too big to fit in the active site of *CYP6P3*. Several studies have revealed that the molecular properties that impact the binding of a substrates are the ligand linear planarity, the Highest Occupied Molecular Orbital (HOMO) energy, Phe residue $\pi - \pi$ stacking, optimum placement of acceptor and donor substituent for interaction with the polar residues in the active site, and desolvation (ClogP) [(65)(66)]. The ligand/molecule planarity (area/depth2 ratio) and molecular mass were established to be the vital requisites for ligand to be particularly P450s substrates/inhibitors. The chemistry of the *F. sycomorus* ligands used in this study showed that they have a relative molecular mass ranging from 198-562 Da.

Compounds 5, 8, and 9 docked well into the active site of *CYP6P3*, whereas compounds 3, 4, 6 and 7 were practically unfit due to steric clashes. Due to the rigid protein target approximation used in Glide programs, the ligands with steric clashes for a specific protein conformation are not good scorers. These ligands are referred to as non-inhibitors of *CYP6P3*. This may be due certain factors, such as, the rotamer side chain that may obstruct ligand atoms from binding to their favored spot in the binding site. The fitting ligands are ranked by Glide XP whereas the unfitting ligands need an induced-fit *modus operandi* [(67)(68)] to suitably assess their binding affinities [(69)].

The best distance linking the substrate/inhibitor atom of oxidation to the heme iron atom (4.0 Å - 7.5 Å range) is regarded as the important yardstick for determining the best mode of inhibitor/substrate binding. The 6.0 Å distance or less between the heme iron and an atom in the substrate/inhibitor were marked as reactive since activation of the C-H bond by the heme-Fe-O reaction complex during catalysis is likely [(70)]. A general method to identify a successful docking pose of P450 is to necessitate that an inhibitor/substrate's site of metabolism (SOM) is within a specific range or distance from the heme central iron in a state of lowest binding energy conformation. Frequently, the maximum distance of 6 Å were suggested [(71)(72)]. A lengthy distance from the heme iron produces a wide area above the heme plane in P450 structures which subsequently allowed room for other inhibitors/substrate atoms besides the SOM. This paves way for error prone process of identifying the SOM due to present of multiple substrate atoms at the active sites.

The P450s have quite number of potentials inhibition stages in their reaction cycle, Viz: substrate binding, ferric (Fe^{3+}) to ferrous (Fe^{2+})(one electron) reduction, oxygen binding to ferrous iron, second electron transfer to the ferrous-oxy-substrate complex and subsequent activated oxygen intermediate and water release, activated oxygen insertion to substrate and release of the oxygenated product and Ferric (Fe^{3+}) form of the P450s [(73)]. The substrate binding, Oxygen binding and transfer of activated oxygen to the substrate stages were more prone to inhibition [(74)]. Several reversible inhibitors act by coordinating with the prosthetic heme iron atom as depicted in figure 3. This

coordination shifts the heme-iron from the high to the low spin form producing a difference spectrum product [75]. The change in the iron spin state occurs concurrently with a change in the redox potential of the P450. This eventually makes reduction by the P450 reductase more difficult [76]. Thus, the inhibition of *CYP6P3* by these ligands is a consequence not only for the occupation of the sixth coordination site of the iron but also the altering the reduction potential of the heme central iron.

These phytoligands may also form quasi-irreversible complexes with the heme central iron-atom. Some inhibitor compounds such as alkyl and aryl methylenedioxyl classes are generally catalyzed by P450 to form intermediate species that tightly coordinate to the prosthetic group forming stable metabolite intermediate complexes (MI complexes) [77]. Another proposed inhibition mechanism of these phytoligands is that they may be oxidized by P450s to an electrophilic reactive intermediate, which forms covalent bonding with the enzyme protein structure causing mechanism-based inhibition [78][79].

Hydrophobic side chain has been proposed to strongly interact with hydrophobic substrate/inhibitor and support water displacement from the active sites during binding [80] as proposed in this study. Consequently, hydrophilic group in the ligands can maintain the iron in low spin state and prevent dislodgment of water molecules from the enzyme active site and thus, inhibiting its activities. The placement of water molecules that are directly involved in binding and the rigidity of side chains can dramatically influence the posing of ligands. And where conformational changes upon binding occur, rigid active sites are limited in their ability to predict poses [81]. This agreed with these study findings as hydrophilic groups from compounds 5, 8 and 9 interact with heme iron and probably maintaining the heme iron in stable low spin state and inhibit oxygen binding.

The computed LE decreases with increasing number of heavy atoms as previously observed by Reynolds et al. [54] and Reynolds et al.[82]. This is contrary to our finding which showed increase in LE with increase of heavy atoms. The contributing factors to the decrease LE for the larger ligands might be due to less favorable binding entropies for larger and flexible compounds [83]. Analysis of large numbers of protein-ligand complexes over a wide range of affinities [84] demonstrates that suitable/optimal, ligand efficiencies are analytically higher for small ligands than large ones. This agreed with the data of this study where compound 5 and 8 showed efficiency greater than 0.3Kcal/mol/HA and thus, can potentially be optimized. Fit Quality (FQ) and SILE convert LE into a metric that is more consistent across wide ranges of molecular size. Similarly, this approach has also been applied to derive size independent enthalpy efficiencies, where free energy is replaced by enthalpy [84]. Analysis of enthalpy and entropy efficiencies showed that the size dependency of a ligand is generally associated with enthalpy [85]. In addition, MD simulation revealed the potentially stable binding mode of compound 5, 8, and 9, thereby further validating both the docking and ligand optimization results.

Conclusion

Compounds 5, 8 and 9 bound to the Heme iron of *CYP6P3* at proximity less than the maximum distance required for reactivity (6 Å). The binding energies indicate non-spontaneous interaction and energy consuming process with the enzyme active site. The most common amino acid residues in the binding pocket were *Phe123, Val310, Pro379 and Val380.* Some reversible and irreversible inhibitors act by coordinating with the prosthetic heme iron atom and formation of quasi-irreversible complexes with the iron of the heme prosthetic group, respectively. The LE matrics showed high potential of these compounds (particularly, compound 5 and 8) to form core fragment for optimization into a potent P450s inhibitors via molecular tactics such as carbon atom replacement and lipophilic group addition. MD simulation performed for these complexes to assess the stability of ligand binding mode. The results show that in addition to being stable in the enzyme binding pocket, these

compounds maintained Fe^{2+}(Heme):O (Ligand) distance over time. Thus, these compounds could serve as potential insecticide synergists and/or provide scaffold for further optimization into potent *CYP6P3* inhibitors.

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Conflicts of interest

None declared

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دراسات النمذجة الجزيئية لبعض النباتات النباتية من جزء Ficus sycomorus كمثبطات محتملة لإنزيم السيتوكروم CYP6P3 من Anopheles coluzzi

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ملخص

العقبة الرئيسية في مكافحة الملاريا هي مقاومة البعوض للمبيدات الحشرية، بما في ذلك البيرثرويد. ترجع المقاومة بشكل أساسي إلى الإفراط في التعبير عن إنزيمات إزالة السموم مثل السيتوكرومات. يمكن تقليل تحمل المبيدات الحشرية عن طريق مثبطات P450s المشاركة في إزالة السموم من المبيدات الحشرية. هنا، لتصميم مثبطات CYP6P3 المحتملة، تم إنشاء نموذج تماثل للإنزيم باستخدام التركيب البلوري للبكتيريا الزرقاء المرتبط بحمض الريتينويك CYP120A1 (معرف PDB: 2VE3 ؛ القرار: 2.1 Å). تم استخدام دراسة الالتحام الجزيئي والنمذجة الحسابية لتحديد الإمكانات المثبطة لبعض النباتات النباتية المعزولة من Ficus sycomorus ضد Anopheles coluzzii النموذجي P450، CYP6P3 ، المتورط في المقاومة. تم تحليل خصائص تحسين الترابط المحتمل (LE) باستخدام النماذج الرياضية القياسية. المركبات 5 و 8 و 9 مرتبطة بحديد Heme لـ CYP6P3 ضمن 3.14 و 2.47 و 2.59 Å، على التوالي. قدرت طاقات الارتباط الخاصة بكل منها بـ −8.93 و −10.44 و −12.56 كيلو كالوري / مول. لفحص ثبات وضع الربط الخاص بهم، تم إخضاع مجمعات الالتحام الناتجة من هذه المركبات باستخدام CYP6P3 لمحاكاة 50 نانوثانية MD. ظلت المركبات مرتبطة بالإنزيم و Fe (Heme): يبدو أن مسافة O (Ligand) قد تم الحفاظ عليها بمرور الوقت. يؤدي التنسيق بين ليجند قوي إلى حديد الهيم إلى تحويل الحديد من الشكل العالي إلى شكل الدوران المنخفض المستقر ومنع الأكسجين من الارتباط بالهيم وبالتالي تثبيط النشاط التحفيزي. أظهر مؤشر LE القدرة العالية لهذه المركبات (5 و 8) لتوفير جزء أساسي لتحسين مثبطات P450 القوية.

الكلمات الدالة: نمذجة التنادد. CYP6P3؛ الالتحام الجزيئي كفاءة يجند محاكاة الديناميات الجزيئية؛ مثبطات CYP6P3.

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Synergistic effects of neem (*Azadirachta indica* L.) leaves extract with conventional antibiotic against gram positive and negative microorganism

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ABSTRACT

Background: Neem has been known to possess several complex phytoconstituent(s) and exhibits wide array of medicinal and household uses that are attributed to its active isolate(s) that possesses an ability to cure many chronic disease(s) and disorder(s). The current investigation aimed to study the combined antimicrobial effect of crude Neem extract and selected antibiotics namely Ciprofloxacin, Cefixime, Chloramphenicol, Ampicillin, Sulfamethoxazole, Tetracycline and Ofloxacin on selected Gram's positive and negative micro organism. Crude alcoholic Neem leaf extract was used for the study.

Method: Solution A containing Neem extract 5 mg mL^{-1} alone, Solution B comprising of Standard antibiotics alone 5 mg mL^{-1} and Solution C containing combination of 2.5 mg mL^{-1} of Neem extract and selected standard antibiotic at a concentration of 2.5 mg mL^{-1} were tested for their antibacterial potential against selected strain of micro-organisms namely *Klebsiella pneumoniae, Staphylococcous aureus, Pseudomonas aeruginosa, E. coli* and *Bacillus subtilis* using agar plate technique.

Results: The obtained results indicated the synergistic activity of the Neem extract and the selected antibiotics. It was observed that half concentration of antibiotic was sufficient to exert the antimicrobial effect when they were combined with Neem extract. Thus, the dose of standard antibiotics may be marginally reduced to almost half in concentration when combined with the Neem extract without compromising the efficacy. The zones of inhibition indicated that the combination of Neem extract and antibiotic exerted a synergistic effect which will facilitate to achieve reduction of the dose of standard antibiotics.

Conclusions: It can be concluded from the research that Neem extract when combined with the conventional antibiotic(s) can be used as a novel antimicrobial agent which exhibits a synergistic effect and also helpful to achieve a dose reduction of conventional antibiotic(s).

Keywords: Neem; Microorganism; Antibacterial; Synergistic effect; Antibiotics.

1. INTRODUCTION

Azadirachta indica L. (Family: Meliaceae) which is commonly known as Neem in India and South East, comprises of several complex plant phytoconstituent(s); and traditionally well known for its numerous medicinal properties and uses. It is a commonly occurring plant known traditionally for its wide array of medicinal uses. It is fast-growing tree that usually attains a height of around 50–72 feet. It is deciduous tree and most of its leaves fall off during the winter season. The branches of the tree are usually broad and extend out with its crown rather thick and roundish, having a diameter of around 65–79 ft. The

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neem tree resembles its close relative, the chinaberry (Melia azedarach). It possesses plentiful medicinal effects and therefore used widely for the treatment of several chronic diseases and disorders.[1]

Owing to its antibacterial, anthelmintic, antifungal properties it has been recommended for treatment and cure of multiple skin diseases.[2] A variety of plant compounds were isolated from the Neem extracts. Quercetin (polyphenolic flavonoid) and ß-sitosterol (phytosteroids) are the prime phytoconstituents that were isolated from fresh Neem leaves which have been reported to exhibit prominent antifungal and antibacterial effects.[3] Studies on 21 isolates from Neem extracts demonstrated efficacy of these compounds against foodborne and spoilage microorganisms.[4, 5] There are also reports about effectiveness of Neem against *Mycobacterium tuberculi*, *Vibrio cholera*e and against some Gram negative and positive bacteria.

Amongst Quinolone antibiotics, Ciprofloxacin and Ofloxacin are of major concern and used widely for the treatment of various bacterial infections, whereas, Cefixime is a Cephalosporin antibiotic of 3rd generation.[6, 7] It exhibits broad and dominant actions against various pathogens, chiefly Gram-negative organisms.[8, 9] Chloramphenicol and Ampicillin, the semi-synthetic moieties are available in the form of orally effective broad-spectrum antibiotics. Sulfamethoxazole is a Sulfonamide of prime importance, whereas, Tetracycline is a wide-spectrum antibiotic. Particularly, these antibiotics have verified superior pharmacokinetic properties and antibacterial actions. The aforesaid antibiotics are used in the treatment of Gram negative and occasionally gram-positive infections.[10-16] These antibiotics have been known to produce several side effects namely diarrhoea, abdominal pain, dizziness, sleepiness, headache, nausea, bad vision, nervousness, anxiety, agitation, insomnia or nightmares, and skin rash.[17, 18] Alternative healthcare systems, such as Ayurveda, Siddha and Unani, have proved their prospective for complementing the healthcare system in India and other developing countries.[19] Moreover, the recent times, have witnessed a greater inclination towards the use of medicinal plants and herbs, owing to their safety and lesser side effect(s) as compared to their allopathic counterparts.[20, 21]

Certain researches have recommended use of antibiotic combinations that have demonstrated a synergistic effect above their individual inhibitory effects. Therefore, in many instances, antibiotics are combined to thwart development of resistance to the agents used alone and also to enjoy a synergistic effect.[22]

Synergistic combinations are often commercially used in the treatment of various infections in antimicrobial chemotherapy. Antibiotic resistance is amongst the supreme threat to the society.[23] Antão and Wagner-Ahlfs have consequently reported that every year more than 700,000 people all over the world lose their lives owing to the drug-resistant infections.[24]

A number of antibiotics used for the treatment of a variety of infectious diseases in humans exhibit limited antimicrobial spectrum particularly due to the emergence of MRD bacterial strains.[25, 26] Thus, it is justified to use two or more antimicrobial agent(s) in combination with intent to holdup or avoid the chances of drug resistant microbial strains. Moreover, in certain instances, the synergistic effect is also observed. Neem is a worldwide recognized natural antibiotic in the ancient Indian system of medicine. Hence, in the present investigation an attempt has been made to study the effect of use of Neem extract along with conventional antibiotics against the selected antibacterial strains.

2. Materials and methods

2.1 Chemicals

Analytical grade chemicals and solvents were used during the entire course of experimental work. The required media and other microbiology accessories were procured from Himedia. The antibiotics used in the experimental work were procured from Research laboratories, Mumbai, Maharashtra.

2.2 Microorganism used

Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and *Bacillus subtilis* were used in the study. The cultures of the aforesaid microorganisms were collected from Yashawantrao Chavan (Y.C) College of Science, Saidapur, Karad (MS) INDIA - 415110.

2.3 Preparation of the crude extracts

In the month of September 2019, the fresh leaves were collected from Kasegaon region, Near National Highway No. 4 (Tal – Walwa, Dist – Sangli, MS – 415404). The taxonomical recognition of the plant material was carried out from Y.C. College Saidapur, Tal – Karad, Dist – Satara, MS – 415110 with (voucher number YC-5). The collected leaves were subjected to drying in a shade for 2 weeks. 250 gm of the dried samples were ground to obtain a coarse powder. Extraction was executed in hot continuous process through Soxhlet apparatus for 48 hours using ethanol (500 mL) as a solvent. Thereafter, the contents of the round bottom flask were filtered through Whatman filter paper and the extract was allowed to evaporate in warm water and dried at room temperature. 22.30 g of the recovered crude neem extract were obtained and the yield was noted to be 8.92% w/w. Condensed extract was weighed and stored in air tight container for 4 °C till further investigation.

2.4 Preparation of Dilution

Preparation of Antibiotic solution – **Solution A:** Ciprofloxacin (50 mg), Cefixime (50 mg), Chloramphenicol (50 mg), Ampicillin (50 mg), Sulfamethoxazole (50 mg), Tetracycline (50 mg) and Ofloxacin (50 mg) were accurately weighed and transferred separately to the labeled test tubes. Thereafter 10 mL of DMSO solution was added to each test tube to obtain a concentration of 5 mg mL^{-1}.

Preparation of the Neem extract solution – **Solution B** was prepared to yield a final concentration of 5 mg mL^{-1} by adding 50 mg of dry Neem extract diluted with 10 mL of DMSO solution.

Preparation of combination (Neem extract and antibiotic solution) (**Solution C**) – It was prepared by adding equal volume of Solution A and Solution B (1 mL each) to get a Solution C containing 2.5 mg mL^{-1} of antibiotic and 2.5 mg mL^{-1} of neem extract separately for each antibiotic.

2.5 Preparation of Agar plate

Nutrient agar is a general-purpose medium that promote the growth of a wide range of micro organisms. It comprises of 0.5% Peptone, 0.3% of beef extract/yeast extract, 1.5% of agar, 0.5% Sodium Chloride and distilled water. All of the above ingredients were combined and boiled for about a minute to facilitate proper mixing and then subjected to sterilization. The contents were then cooled at about 50 °C (122 °F). The resultant liquid was then transferred into labeled petri plates and the cap was immediately secured. Once the dishes grip solidified agar, they were stored upside down and were refrigerated until used.

2.6 Preparation of test organism

Normal agar plates were inoculated with respective bacteria namely *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*, thereafter incubated at 37° C for overnight. Each time, a fresh bacterial culture was prepared.

2.7 Antimicrobial activity

Antimicrobial activity (*In-vitro)* was determined using the Agar well diffusion technique. [21, 27, 28] Sterile agar was inoculated with the bacterial culture of *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* for 48 h, at 37ºC. Antimicrobial activities of solution A, B and C were tested on nutrient medium against *Klebsiella Pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* which are representative types of Gram-positive and Gram-negative microorganisms. Sterile borer was used to make wells in the poured culture containing microrganisms and 50 µL of solution A, B and C were added into it. Thereafter, the

plates were kept for 2 hours in a refrigerator to enable pre-diffusion of the solution A, B and C. Finally, the plates containing solution A, B and C were incubated overnight (24 hours, at 37ºC). The antimicrobial activity was assessed by measurement of the diameter of zone of inhibition.

3. Results

Test results revealed that the combination of Neem extract with the antibiotic exerts a synergistic effect thereby reducing the dose of the conventional antibiotics (**Figure 1**, **2**, **3**, **4** and **5**). Antimicrobial activity was assessed by measuring the diameter of zone of inhibition. The outcomes in the assessment of the antimicrobial activity of Solution A, Solution B and Solution C are noted in **Table 1**. Solution C which comprised of Neem extract along with Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline (1:1) showed zone of inhibition values as 21.00±1.5000, 16.90±1.8520, 13.10±0.3605, 39.83±0.7637, 39.83±0.7637, 26.43±0.9073 and 23.13±1.1060 respectively against the *Klebsiella pneumoniae.* Whereas, solution A which comprised of standard antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline at a concentration of 5 mg mL^{-1} exhibited zone of inhibition as 21.91±0.6291, 20.00±1.0000, 18.06±0.4041, 40.03±1.1503, 40.03±1.1503, 25.43±1.2096 and 25.16±0.6658, respectively. For *Staphylococcous aureus* the zone of inhibition was observed to be 38.10±0.3605, 45.13±0.7094, 15.93±0.5033, 42.23±0.3214, 37.76±0.8504, 35.30±1.3114 and 42.50±0.9165 for solution C which comprised of Neem extract along with standard antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline respectively. Solution A which comprised of standard antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline exhibited the zones of inhibition to be 42.00±0.5011, 41.93±1.9008, 15.86±0.5131, 47.46±1.5502, 38.23±0.7094, 35.66±0.7371, and 39.66±1.4294 respectively against *Staphylococcous aureus*. Further, Solution C which comprised of Neem extract along with Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline separately (1:1) showed zone of inhibition values to be 19.10±0.6557, 13.46±0.568624, 7.20±0.2645, 32.63±1.0692, 42.23±0.3214, 34.87±0.9073, and 22.93±0.5131 respectively against *Pseudomonas aeruginosa.* While solution A which contained standard antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline at a concentration of 5 mg mL^{-1} exhibited zone of inhibition to be 17.73±0.4041, 15.00±0.4001, 7.10±0.3605, 33.76±0.8736, 41.87±0.7094, 35.00±0.9005 and 30.26±0.9609. Whereas the zone of inhibition for Chloramphenicol, Ampicillin, Sulfa methoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline for solution C (Antibiotics and Neem extract) was observed to be 25.46±0.8962, 13.00±0.4001, 11.96±0.3511, 33.30±1.0816, 33.30±1.0816, 23.10±1.2489, and 28.50±0.5567 against *Bacillus subtilis.* While solution A (Standard antibiotic) containing Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline separately noted zone of inhibition to be 27.16±0.5686, 10.86±0.4163, 14.90±0.5567, 33.93±0.7023, 33.93±0.7023, 23.37±0.7023 and 30.63±0.4725, respectively.

In case of *E. coli*, the zone of inhibition was observed to be 32.46±0.8962, 23.43±0.5033, 15.70±0.8001, 33.13±0.9073, 35.13±1.1239, 20.33±0.7681, and 26.85±0.5766 for solution C containing neem extract along with standard antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline respectively. Solution A containing plain antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline showed the zone of inhibition as 35.90±0.9539, 25.97±0.7371, 20.43±0.7571, 33.63±1.2055, 38.60±0.8185, 25.73±1.0692 and 34.60±1.4525, respectively against the *E. coli*.

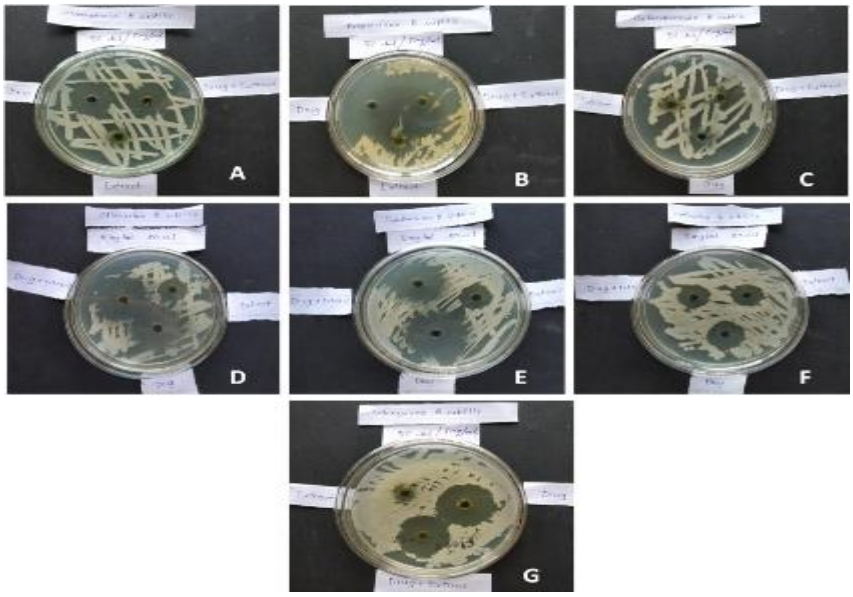


Figure 1. Photograph of Antimicrobial activity of A) Chloramphenicol B) Ampicillin C) Sulfamethoxazole D) Ofloxacin E) Ciprofloxacin F) Cefixime G) Tetracycline against *Bacillus subtilis*

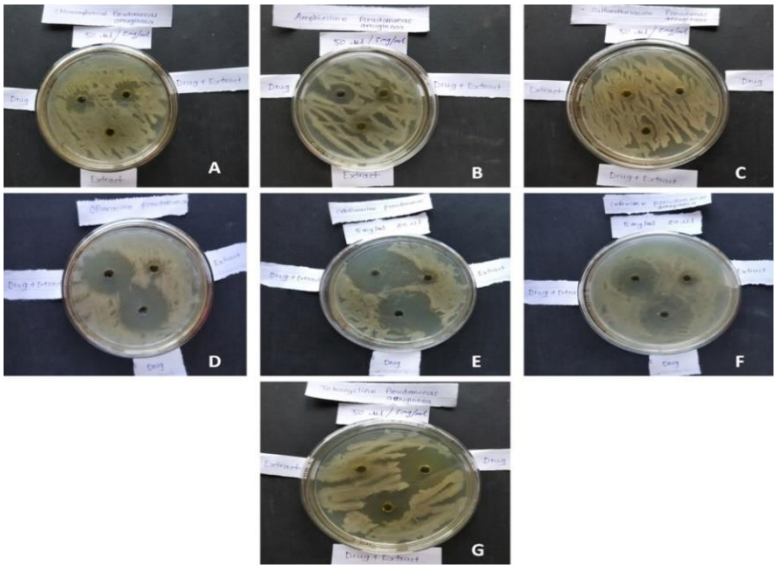


Figure 2. Photograph of Antimicrobial activity of A) Chloramphenicol B) Ampicillin C) Sulfamethoxazole D) Ofloxacin E) Ciprofloxacin F) Cefixime G) Tetracycline against *Pseudomonas aeruginosa*

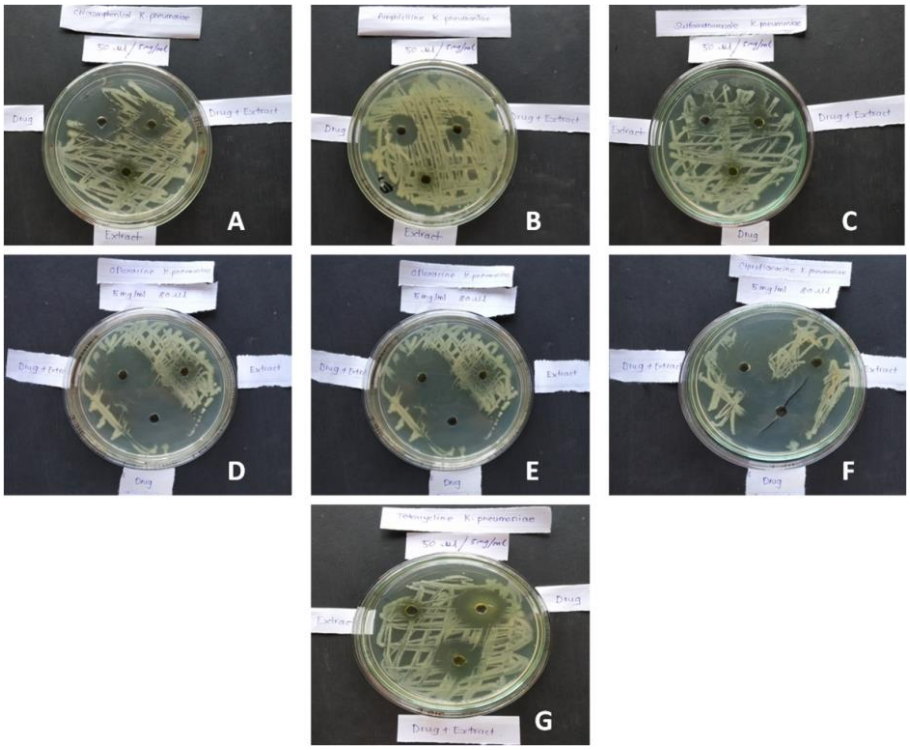


Figure 3. Photograph of Antimicrobial activity of A) Chloramphenicol B) Ampicillin C) Sulfamethoxazole D) Ofloxacin E) Ciprofloxacin F) Cefixime G) Tetracycline against *Klebsiella Pneumoniae*

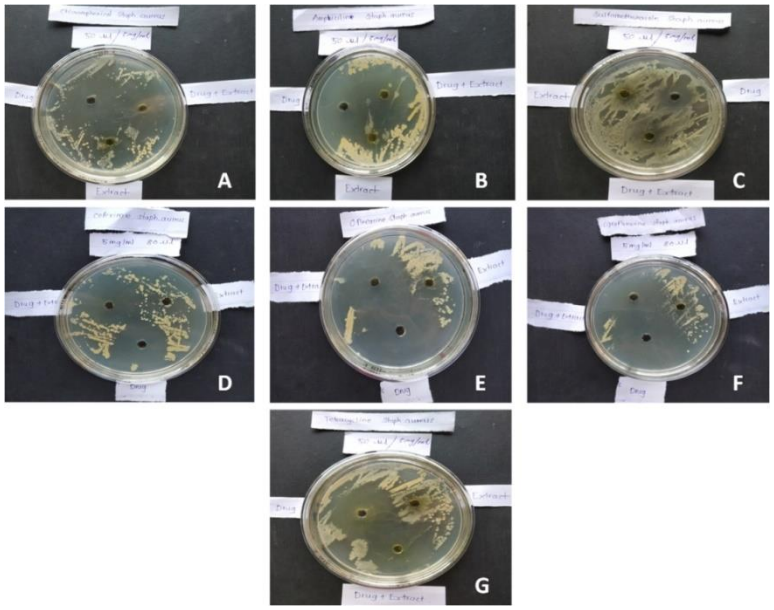


Figure 4. Photograph of Antimicrobial activity of A) Chloramphenicol B) Ampicillin C) Sulfamethoxazole D) Ofloxacin E) Ciprofloxacin F) Cefixime G) Tetracycline against Staphylococcous aureus

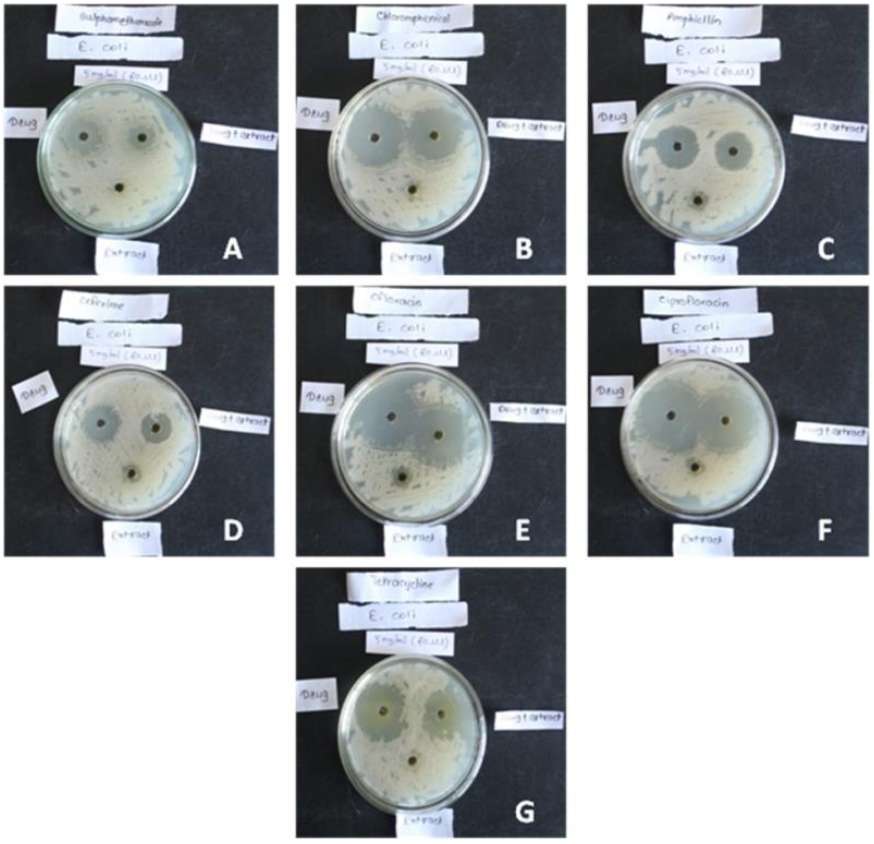


Figure 5. Photograph of Antimicrobial activity of A) Chloramphenicol B) Ampicillin C) Sulfamethoxazole D) Ofloxacin E) Ciprofloxacin F) Cefixime G) Tetracycline against *E-coli*

Table 1. Effect of selected antibiotics (Solution A), neem extract (Solution B) and selected antibiotics+neem extract (Solution C) against the *Klebsiella Pneumoniae*, *Staphylococcous aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*

Sr. No.	Microorganism	Solution A (zone inhibition in mm ±SEM)[x]	Solution B (zone inhibition in mm ±SEM)[y]	Solution C (zone inhibition in mm ±SEM)[x]
		***Klebsiella Pneumoniae*[b]**		
1.	Chloramphenicol	21.91±0.6291	13.53±1.6772	21.00±1.5000
2.	Ampicillin	20.00±1.0000		16.90±1.8520
3.	Sulfamethoxazole	18.06±0.4041		13.10±0.3605
4.	Ofloxacin	40.03±1.1503		39.83±0.7637
5.	Ciprofloxacin	40.03±1.1503		39.83±0.7637
6.	Cefixime	25.43±1.2096		26.43±0.9073
7.	Tetracycline	25.16±0.6658		23.13±1.1060

Sr. No.	Microorganism	Solution A (zone inhibition in mm ±SEM)[x]	Solution B (zone inhibition in mm ±SEM)[y]	Solution C (zone inhibition in mm ±SEM)[x]
		Staphylococcous aureus[a]		
1.	Chloramphenicol	42.00±0.5011		38.10±0.3605
2.	Ampicillin	41.93±1.9008		45.13±0.7094
3.	Sulfamethoxazole	15.86±0.5131		15.93±0.5033
4.	Ofloxacin	47.46±1.5502	12.36±2.6674	42.23±0.3214
5.	Ciprofloxacin	38.23±0.7094		37.76±0.8504
6.	Cefixime	35.66±0.7371		35.30±1.3114
7.	Tetracycline	39.66±1.4294		42.50±0.9165
		Pseudomonas aeruginosa[b]		
1.	Chloramphenicol	17.73±0.4041		19.10±0.6557
2.	Ampicillin	15.00±0.4001		13.46±0.5686
3.	Sulfamethoxazole	7.10±0.3605		7.20±0.2645
4.	Ofloxacin	33.76±0.8736	9.53±0.9890	32.63±1.0692
5.	Ciprofloxacin	41.87±0.7094		42.23±0.3214
6.	Cefixime	35.00±0.9005		34.87±0.9073
7.	Tetracycline	30.26±0.9609		22.93±0.5131
		Bacillus subtilis[a]		
1.	Chloramphenicol	27.16±0.5686		25.46±0.8962
2.	Ampicillin	10.86±0.4163		13.00±0.4001
3.	Sulfamethoxazole	14.90±0.5567		11.96±0.3511
4.	Ofloxacin	33.93±0.7023	13.75±1.3871	33.30±1.0816
5.	Ciprofloxacin	33.93±0.7023		33.30±1.0816
6.	Cefixime	23.37±0.7023		23.10±1.2489
7.	Tetracycline	30.63±0.4725		28.50±0.5567
		E-coli[b]		
1.	Chloramphenicol	35.90±0.9539		32.46±0.8962
2.	Ampicillin	25.97±0.7371		23.43±0.5033
3.	Sulfamethoxazole	20.43±0.7571		15.70±0.8001
4.	Ofloxacin	33.63±1.2055	8.72±1.1537	33.13±0.9073
5.	Ciprofloxacin	38.60±0.8185		35.13±1.1239

Sr. No.	Microorganism	Solution A (zone inhibition in mm ±SEM)^x	Solution B (zone inhibition in mm ±SEM)^y	Solution C (zone inhibition in mm ±SEM)^x
6.	Cefixime	25.73±1.0692		20.33±0.7681
7.	Tetracycline	34.60±1.4525		26.85±0.5766

Note – a indicate Gram Positive Bacteria, b – indicate Gram Negative Bacteria, x – indicate mean of 3 readings, y – indicate mean of 21 readings

4. Discussion

In the present era, the use of herbal medicines is ever increasing owing to their minimal side effect(s) as compared to their allopathic counterparts.

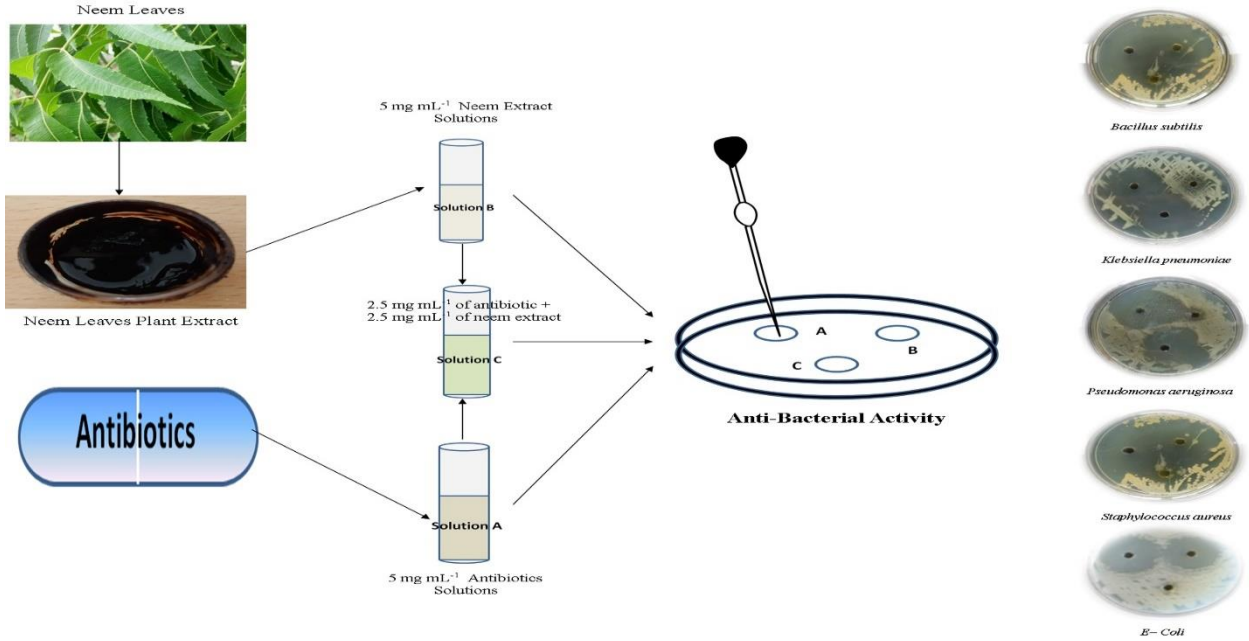
Since ancient times, these medicinal herbs have been used for the treatment of various diseases and disorders because of their effectiveness and nominal side effect(s). The Indian traditional medicine system, Ayurveda presents a classic example which describes herbal medicines for the prevention as well as treatment of several forms of human illnesses.

A range of human infections are treated by the several antibiotics and one of the approach for prevention or delaying of development of resistant microbial strains is to employ combination therapy of antimicrobials. Another prominent reason is that in some cases antibiotic combinations may lead to synergism, particularly in other-resistant cases, which prove to be of help for the treatment of infections. The combined administration of herbs / herbal extracts has also been shown to be beneficial (synergistic or additive) or deleterious in patient antibiotic therapy and that implies a possible herb-drug interaction (antagonistic or toxic outcome). Several side effect(s) previously discussed have been reported by antibiotics. Effective antibiotics with no or minimal side-effect(s) are therefore urgently needed. Neem is known worldwide for its excellent antimicrobial properties. Therefore, we have verified the antimicrobial activity of a combination of some conventional antibiotics with Neem extract.

We have tried to find out effect of use of Neem extract with standard antibiotics with intent to decrease the dose of standard antibiotic. The standard antibiotic dose was reduced to half when combined with Neem extract, without affecting its effectiveness, as demonstrated in the study.

Neem extract alone exhibited zone of inhibition of 13.53±1.6772, 12.36±2.6674, 9.53±0.9890, 13.75±1.3871 and 8.72±1.1537 against selected microbial strains namely, *Klebsiella Pneumoniae, Staphylococcous aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *E.coli* respectively. In case of *Klebsiella Pneumoniae,* the combination of Cefixime and neem exhibited zone of inhibition of 26.43±0.9073, as compared to Cefixime alone which showed zone of inhibition of 25.43±1.2096. For *Staphylococcous aureus,* 45.13±0.7094 was observed to be the zone of inhibition in case of combination of Ampicillin and Neem, which was relatively better than Ampicillin alone which showed 41.93±1.9008 as zone of inhibition. Similarly, combination of Tetracycline and Neem offered better inhibition of 42.50±0.9165, than Tetracycline alone against *S.aureus*. Also, the combination of Neem and Chloramphenicol showed better zone of inhibition of 19.10±0.6557 as compared to Chloramphenicol alone against *Pseudomonas aeruginosa.* In addition, the combination of Ciprofloxacin and Neem showed better efficacy than

Ciprofloxacin alone against the said organism. For Bacillus subtilis, 13.00±0.4001 was noted to be the zone of inhibition for combination of Neem and Ampicillin than Ampicillin alone which showed zone of inhibition of 10.86±0.4163. However, in case of E. coli the values of zones of inhibition of the selected antibiotics were



comparable with that of their combination with Neem extract.

The aforementioned results of solution A and solution C clearly demonstrate the synergistic activity of Neem extracts when combined standard antibiotics as observed in the values of zone of inhibition. Thus, Neem extract and antibiotic combination significantly minimize the standard dose of antibiotics.

5. Conclusions

As compared with their combination with the antibiotics, the individual plant extract have been less efficient. According to our study a synergistic effect have been observed with the use of combination of Neem extract and antibiotic against the selected microbial strains which is an important finding of this work. This synergism may be due to complex formation that is more effective in destruction of microbial cell, either through inhibition of cell wall synthesis or its lysis. Furthermore, the research showed that Neem extract can be used with conventional antibiotics as this combination exhibited a synergistic effect that may lead to reduction in dose of the antibiotics.

Abbreviations

MDR - Multidrug-resistant; *K. pneumonia - Klebsiella pneumonia*; *P. aeruginosa - Pseudomonas aeruginosa*; *S. aureus - Staphylococcus aureus*; *E. coli - Escherichia coli*; *B. subtilis - Bacillus subtilis;* DMSO – Dimethylsulfoxide; ^{o}C – Degree Celsius; µL – micro liter; mL – mille liter

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التأثيرات التآزرية لمستخلص أوراق النيم مع المضاد الحيوي التقليدي ضد الكائنات الدقيقة الإيجابية والسلبية للجرام

سوموناث دز [1*]، ديراج س. راننديب [1]، مانجيش أ بوتكارب [1]، كيران ب شجولب [1]، أميت د. جادافا [1]، راهول ب. جادهافا [1]

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ملخص

الخلفية: من المعروف أن النيم يمتلك العديد من المكونات النباتية المعقدة ويعرض مجموعة واسعة من الاستخدامات الطبية والمنزلية التي تُعزى إلى العزلة (العزلات) النشطة التي تمتلك القدرة على علاج العديد من الأمراض المزمنة والاضطرابات (الاضطرابات). هدفت الدراسة الحالية إلى دراسة التأثير المشترك المضاد للميكروبات لمستخلص النيم الخام والمضادات الحيوية المختارة مثل سيبروفلوكساسين، سيفيكسيم، كلورامفينيكول، أمبيسلين، سلفاميثوكسازول، تتراسيكلين وأوفلوكساسين على الكائنات الدقيقة الإيجابية والسلبية للجرام المختار. تم استخدام مستخلص أوراق النيم الكحولي الخام للدراسة.

الطريقة: المحلول أ يحتوي على مستخلص النيم 5 مجم مل 1 وحده، المحلول ب يتكون من المضادات الحيوية القياسية وحدها 5 مجم مل 1 ومحلول ج الذي يحتوي على توليفة من 2.5 مجم مل من مستخلص النيم ومضاد حيوي معياري محدد بتركيز 2.5 مجم تم اختبار mL-1 من حيث قدرتها المضادة للبكتيريا ضد سلالة مختارة من الكائنات الدقيقة مثل Klebsiella pneumoniae و Staphylococcus aureus و Pseudomonas aeruginosa و E. coli و Bacillus subtilis باستخدام تقنية لوحة أجار.

النتائج: أشارت النتائج المتحصل عليها إلى الفعالية التآزرية لمستخلص النيم والمضادات الحيوية المختارة. لوحظ أن نصف تركيز المضاد الحيوي كان كافياً لممارسة التأثير المضاد للميكروبات عند مزجها مع مستخلص النيم. وبالتالي، قد يتم تقليل جرعة المضادات الحيوية القياسية بشكل هامشي إلى النصف تقريبًا في التركيز عند دمجها مع مستخلص النيم دون المساس بالفعالية. أشارت مناطق التثبيط إلى أن الجمع بين مستخلص النيم والمضاد الحيوي لهما تأثير تآزري يسهل تحقيق خفض جرعة المضادات الحيوية القياسية.

الاستنتاجات: يمكن الاستنتاج من البحث أن مستخلص النيم عند دمجه مع المضادات الحيوية التقليدية يمكن استخدامه كعامل مضاد للميكروبات جديد له تأثير تآزري ومفيد أيضًا في تحقيق تقليل جرعة المضادات الحيوية التقليدية.

الكلمات الدالة: نيم؛ الكائنات الحية الدقيقة. مضاد للجراثيم. تأثير تآزري مضادات حيوية.

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A Comprehensive Review on Efficacy and Adverse Events Associated With Different Covid-19 Vaccines

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ABSTRACT

To combat COVID-19, various health agencies around the world gave emergency approval for vaccines. Therefore, the long-term protective effect and the potential adverse effects of the vaccines on immunocompromised patients, pregnant women and geriatrics might not be well-established. The aim of this review was to assess the safety and efficacy of a number of the most commonly approved vaccines all over the world. A review was made to identify clinical trials that studied the vaccines' efficacy and case reports of potential suspected vaccine-related adverse events. The electronic databases searched to identify relevant studies were Science Direct, PubMed/Medline, Scopus and MedRxiv. Seven randomized controlled trials which assessed the efficacy of COVID-19 vaccines and case reports which reported the vaccines' adverse events were included in the review. The efficacy of the vaccines was found to be 94.6% for Pfizer vaccine, 94.1% for Moderna vaccine, 66.1% for Johnson and Johnson's, 76.4% for Covishield, 91.6% for Sputnik, and 77.8% for Covaxin. No severe adverse events were reported in the studies. All the reported adverse events were mild, self-sustaining and did not require any medical intervention. All the COVID-19 vaccines demonstrated promising immunogenicity profile, different degrees of protective effectiveness and a tolerable safety profile. However, further research to evaluate the efficacy and safety in vulnerable populations including immunocompromised patients, pregnant women and geriatric populations are needed. The long term post marketing surveillance becomes a very important part of identifying the efficacy and side effects among different populations.

Keywords: COVID-19 Vaccine, Vaccine Efficacy, Vaccine Adverse effects.

INTRODUCTION

The novel human viral pathogen severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was identified as the source of the coronavirus disease 2019 (COVID-19) pandemic in Wuhan, China, in late 2019 [1]. As a major worldwide public health event, the COVID-19 outbreak has become the world's principal health concern, having a significant political, economic, and cultural influence. COVID-19 causes fever and a dry cough, as well as damage to various organs, particularly the lungs. The use of a mask and keeping social distance has been established as one of the most effective ways to prevent the virus from spreading, and isolation and symptomatic supportive therapy continue to be the most common treatments for COVID-19 patients.

To contain the COVID-19 pandemic, a protective vaccine will be required in order to achieve sufficient herd immunity against SARS-CoV-2 infection. According to the World Health Organization (WHO), more than 200 COVID-19 vaccines are being developed [2]. The effectiveness of vaccinations in preventing disability and mortality from other infectious illnesses validates the belief that COVID-19 may

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be controlled by preventive immunisation. Vaccines against infectious illnesses are expected to save at least 23 million lives between 2011 and 2020.

When emergency approval for COVID-19 vaccines is granted due to high necessity, it is important to demonstrate the vaccines' safety [2]. Identifying, measuring, and balancing known and speculated safety concerns against possible benefits is a crucial part of the vaccine development process. This is notably essential for protecting individuals who are at a higher risk of severe illness from COVID-19, such as healthcare providers, older or elderly adults, and people with other chronic illnesses.

In this review, we will discuss the efficacy and adverse events of a few of the most commonly approved vaccines around the world, i.e, Pfizer, Moderna, Johnson and Johnson, AstraZeneca/Oxford Vaccine, Sputnik, Covishield and Sinopharm vaccines.

Materials and Methods

Search Strategy:

This review was carried out according to the "Preferred Reporting Project for Systematic Evaluation and Meta-Analysis" criteria" (PRISMA) shown in Figure 1.To identify the clinical trials evaluating COVID-19 vaccines and case reports regarding adverse events of COVID-19 Vaccine, Databases searched for data until June 2021 were Science Direct, PubMed/Medline, Scopus and MedRxiv were systematically screened for medical literature.

The searching strategy(s) employed were “AZD1222, SARS-CoV-2, Covid-19, BNT162b1, mRNA-1273, rAd26, rAd5, AD5-nCOV, Ad26.COV2, BBIBP-CorV, BBV152 and CoronaVac”.

Literature Inclusion Criteria:

1. Randomised Controlled Trials in phase I/II/III of COVID-19 vaccines were included.
2. Case reports reporting the suspected adverse events due to COVID-19 vaccines were included.

Literature Exclusion Criteria:

1. Non-RCT studies, research without a control group, preclinical studies, animal phase studies, meta-analyses, letters to the editor, studies with no extractable data, and news items were all excluded from this study.
2. One of the two studies that overlapped was eliminated;
3. Articles published in Languages other than English were eliminated.

Data Extraction:

Data was extracted from the papers by three independent reviewers. First authors, published year, vaccine name, company, study type, vaccine type, trial phase, injection interval (days), trial country, all adverse events, and efficacy-related data were all gathered from each publication. Two authors extracted data separately, while a third author randomly evaluated the collected data.

The vaccine's safety and efficacy were among the key findings of this review. Local adverse reactions and systemic adverse reactions were used to evaluate safety. Geometric mean titres (GMT) of SARS-CoV-2, seroconversion rate, and the reaction of IgG or other specific antibodies to the receptor binding site of the SARS-CoV-2 spike protein were all used as immunogenicity markers

Literature Search Flowchart:

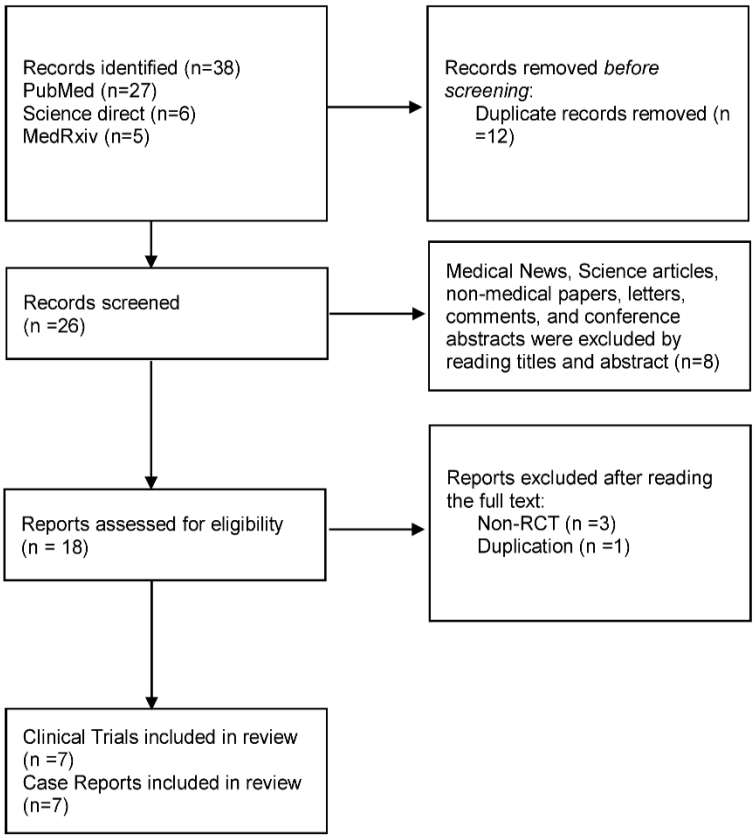

Figure 1

Results

PFIZER/BIONTECH (BNT162b2)

Efficacy:

The findings of the phase II/III trial, released on December 31, 2020, showed that a two-dose regimen of the Pfizer vaccine provides 94.6% protection against symptomatic COVID-19 for at least 7 days after the second dose. There was only one incidence of severe COVID-19 in the vaccination group, compared to nine in the placebo group (Table-1). According to data from an observational research involving 7,000 Israeli healthcare workers, vaccination effectiveness against symptomatic COVID-19 infection was 85 percent after 15–28 days from the first dose. [20]

Table1:
Efficacy of Various Vaccines from Clinical Trials:

Author Name(s)	Vaccine	Vaccine Type	Mechanism Of Immune Stimulation	Sample Size	Study Site(s)	Adverse Effects	Overall Efficacy	Emergency Use Authorization
F.P. Polack, et al. December 2020 [3]	BNT162b2 mRNA Covid-19 Vaccine (Pfzier)	Nucleoside-modified RNA vaccine	mRNA induces cells to produce spike proteins which trigger antibody production.	Total Size-43,448 Dose-1 Vaccine-18,860 Placebo-18,846 Dose-2 Vaccine-18,556 Placebo-18,530	Argentina Brazil United States	Fatigue and headache (59% and 52%, respectively)	94.6%	Approved in several countries. Emergency use in USA, EU, UK, Bahrain, Canada, Saudi Arabia, Mexico, etc.
L.R. Baden, et al. December 2020 [4]	mRNA-1273 (Moderna)	Lipid nanoparticle (LNP)-encapsulated mRNA vaccine.	mRNA induces cells to produce spike proteins which trigger antibody production.	Total Size-30,351 Vaccine-15,181 Placebo-15,170	United States	Injection site pain (91.6%), fatigue (68.5%), headache (63.0%), muscle pain (59.6%), joint pain (44.8%)	94.1%	Approved in Switzerland. Emergency use in USA, UK and EU
Denis Y Logunov, et al.[5]	Gam-COVID-Vac (Sputnik V)	Heterologous recombinant adenovirus (rAd)-based vaccine	Induce antigen-specific cellular immunity (specific T-cell immunity)	Total Size-19,866 Vaccine-14964 Placebo-4902	Russia	Pain at the site of injection (58%), headache (42%), hyperthermia (50%), asthenia (28%) and muscle and joint pain 18 of 76 (24%).	91.1%	Early use in Russia
J. Sadoff, et al. January 2021[6]	Ad26.COV2.S Vaccine (Johnson and Johnson)	Recombinant, replication-incompetent human adenovirus type 26 (Ad26) vector	Induce antibody-based immune responses	Total Size-43,783 Vaccine-21,895 Placebo-21,888	Argentina Brazil Chile Colombia Mexico Peru South Africa United States.		66.1%	Emergency use in USA and Bahrain

Author Name(s)	Vaccine	Vaccine Type	Mechanism Of Immune Stimulation	Sample Size	Study Site(s)	Adverse Effects	Overall Efficacy	Emergency Use Authorization
Merryn Voysey, et al December 2020 [7]	ChAdOx1 nCoV-19 vaccine (Covishield)	Viral vector (Non-replicating)	Induce anti-spike IgG responses and spike-specific T-cell responses.	UK-1 Total Size-2741 Vaccine-1376 Placebo-1374 UK-2 Total Size-4807 Vaccine-2377 Placebo-2430 Brazil Total Size-4088 Vaccine-2036 Placebo-2025	UK Brazil	Fatigue and headache	76.4%	UK, Argentina, El Salvador, Dominican Republic, India, Bangladesh, Mexico, Nepal, Pakistan, Brazil, Saudi Arabia, Iraq, Hungary, Thailand
Shengli Xia, et al January 2021 [8]	BBIBP-CorV Vaccine (Sinopharm)	Inactivated vaccine	Antibody-based immune responses	China Total Size-448 Vaccine-336 Placebo-112	China	Pain and fever	Day 14 seroconversion rates: 97.6%	China, Bahrain, United Arab Emirates, Egypt, Jordan, Iraq, Pakistan, Serbia.
Ella, et al. March 2021[9]	BBV152(Covaxin)	Inactivated Vaccine	Humoral and Immune mediated responses.	Total Size-370	India	Pain and Fatigue	77.8%	India

Table 2:
Covid Vaccine Adverse Events in Case Reports:

Author, Publication Year	Vaccine	Main Diagnosis	Age and Co-Morbidities	Presenting Symptoms	Outcome	Proposed Pathophysiology
Omar Fueyo-Rodriguez. 2021[10]	BNT162b2 mRNA Covid-19 Vaccine(Pfizer)	Immune thrombocytopenia	41 Year/F Multiple Allergies (Quinolones, Cephalosporins)	Fever, tachycardia and nausea.	Patient Improved	Molecular mimicry
Erika Z. Lopatynsky-Reyes. 2021[11]	BNT162b2 mRNA Covid-19 Vaccine (Pfizer)	BCG Scar Local Skin Inflammation	31 year old/F Nil	Headaches, chills, and myalgias in the upper and lower limbs.	Patient Improved on his own.	delayed inflammatory reactions (DIR) to HA fillers
Erika Z. Lopatynsky-Reyes. 2021[11]	mRNA-1273 (Moderna)	BCG Scar Local Skin Inflammation	28 year old/F Nil	headache, nausea, myalgias, arthralgias, and malaise lasting for two days	Patient Improved on his own.	delayed inflammatory reactions (DIR) to HA fillers
Shreena Umit Patel. 2021[12]	ChAdOx1 nCoV-19 vaccine (Covishield)	Guillain-Barre syndrome	37 year old/M Nil	2 weeks following the vaccination, the patient developed persistent back pain, new onset of distal paraesthesia within the hands and feet alongside a symmetrical, progressive ascending muscle weakness	Patient was treated with IVIG. His neurological symptoms have been slow to recover and he is currently under observation in a quaternary neurology centre.	Molecular mimicry response (antibodies against neuronal myelin sheaths and resulting in GBS).
Josef Finsterer. 2021[13]	Covaxin	Guillain-Barre syndrome	32 Year old/ M Nil	Developed paraesthesia on both foot soles two days prior to admission followed by paraesthesias of both palms and dysphagia one day later. Additionally, he reported bilateral frontal and nuchal headache on day-1	-	-
Jackie M Helms. 2021[14]	mRNA-1273 (Moderna)	Immune Thrombocytopenic Purpura	72 Year Old/M Hypertension, gout, hyperlipidaemia and nonischaemic cardiomyopathy	Acute epistaxis and diffuse cutaneous purpura a few hours after receiving the first dose of the Moderna SARS-CoV2 vaccine.	The patient was treated with prednisolone, romiplostim and plasma exchange.	-
Larissa Lebedev. 2021[15]	BNT162b2 mRNA Covid-19 Vaccine(Pfizer)	Minimal Change Disease	50 year Old/ M Nil	On the third day after the injection, he developed abdominal pain and diarrhoea. One day later, he noticed swelling of the lower extremities, which gradually worsened over the next 6 days.	The patient was treated with steroids and anti-hypertensives.	T cell–mediated podocyte injury.

Author, Publication Year	Vaccine	Main Diagnosis	Age and Co-Morbidities	Presenting Symptoms	Outcome	Proposed Pathophysiology
Elisabeth Albert. 2021[16]	mRNA-1273 (Moderna)	Myocarditis	24 Year Old/ M Nil	The patient experienced subjective fever, chills, and body aches in the first 24 hours after the shot. His symptoms progressed to a substernal chest pain, which was exacerbated with deep inspiration and supine position.	-	-

Table 3:
COVID Vaccine Side effects Profile according to CDC in people aged 18-55 years:

Vaccine	Total Size	Dose-1		Dose-2	
		Any Systemic Adverse Reactions	Systemic Adverse Reactions	Any Systemic Adverse Reactions	Systemic Adverse Reactions
Pfizer Vaccine[17]	Dose-1 = 2291 Dose-2 = 2098	82.8%	Headache-41.9% Fatigue-47.4% Chills-14% Myalgia-21.3% Arthralgia-11% Diarrhea-11.1%	82.8%	Headache-51.7% Fatigue-59.4% Chills-35.1% Myalgia-37.3% Arthralgia-21.9% Diarrhea-10.4%
Moderna Vaccine[18]	Dose-1 = 11405 Dose-2 = 10358	57%	Headache-35.4% Fatigue-38.5% Myalgia-23.7% Arthralgia-16.6% Nausea / Vomiting-9.3% Chills-9.2%	81.9%	Headache-62.8% Fatigue-67.6% Myalgia-61.3% Arthralgia-45.2% Nausea / Vomiting-21.3% Chills-48.3%
Janssen COVID-19 vaccine[19]	Total-2036	61.5%	Fatigue-43.8% Headache-44.4% Myalgia-39.1% Nausea-15.5% Fever-12.8%	-	-

Table4:
COVID Vaccine Side effects Profile according to CDC in persons aged >55 years:

Vaccine	Total Size	Dose-1		Dose-2	
		Any Systemic Adverse Reactions	Systemic Adverse Reactions	Any Systemic Adverse Reactions	Systemic Adverse Reactions
Pfizer Vaccine[17]	Dose-1=1802 Dose-2=1660	70.6%	Fatigue-34.1% Headache-25.2% Chills-6.3% Diarrhoea-8.2% Myalgia-13.9% Arthralgia-8.6%	70.6%	Fatigue-50.5% Headache-39% Chills-22.7% Diarrhoea-8.3% Myalgia-28.7% Arthralgia-18.9%
Moderna Vaccine[18]	Dose-1=3761 Dose-2=3589	48.3%	Headache-33.3% Fatigue-38.5% Myalgia-19.8% Arthralgia-16.4% Nausea / Vomiting-5.2% Chills-5.4%	71.9%	Headache-46.4% Fatigue-58.4% Myalgia-46.9% Arthralgia-34.9% Nausea/Vomiting-11.8% Chills-30.6%
Janssen COVID-19 vaccine[19]	Total-1320	45.3%	Fatigue-29.7% Headache-30.4% Myalgia-24% Nausea-12.3% Fever-3.1%	-	-

Additionally, observational data from a U.S. study compiled by the U.S. Centres for Disease Control and Prevention (CDC), reported that mRNA vaccines (Pfizer and Moderna) efficacy was 80% at least after one day from the first dose and 90% at least after one day from the second dose for asymptomatic and symptomatic infections. [21]

Alpha (B.1.1.7) and Beta (B.1.351) - Observational data from Qatar stated that vaccine efficacy was 89.5% in preventing symptomatic COVID-19 for a minimum of 14 days. Vaccine efficacy against severe, critical, or fatal illnesses caused by Alpha or Beta variants was 97.4%. [22]

Delta (B.1.617.2) - An observational study conducted by Public Health England, found that the Pfizer/BioNTech vaccine has an efficacy of 87.9% against symptomatic disease from the B.1.617.2 variant 2 weeks after the second dose. Vaccine efficacy against the B.1.617.2 variation was 33.2% after one dose. [23]

Geriatric Population: An earlier observational study reported that the population over 85 years old were found to have 94.1% protection against Symptomatic COVID-19, 96.9% protection against hospitalization, and 97% protection against death after seven days from receiving the second vaccine dose. [24]

Adverse effects:

The Phase II/III research found that people who took Pfizer vaccine demonstrated transitory adverse effects more frequently than placebos.

Fever, cough, tiredness, headache, shortness of breath, chills, muscular discomfort, sore throat, diarrhoea, or vomiting were the most often reported occurrences after months follow-up.(Table-4)

Updates:

On August 23, 2021, the FDA has approved the use of Pfizer-BioNTech COVID-19 Vaccine, marketed as COMIRNATY, for the prevention of COVID-19 in children above 16 years of age. [36] As of October 29, 2021, the vaccine has been approved for emergency use in children 5 through 11 years of age. [37] The FDA had issued an EUA for the vaccine on November 22, 2021, for the use of a 3rd primary series dose for immunocompromised individuals above the age of 12 years. [38]

MODERNA (mRNA-1273)

Efficacy:

The findings of a phase III trial released on February 4, 2021 show that a complete regimen of Moderna provided 94.1% protection against symptomatic COVID-19 for a minimum of 14 days after complete vaccination. (Table1)

Observational data from a US study published by the Centers for Disease Control and Prevention reported that mRNA vaccine (Pfizer/Moderna) efficacy against COVID-19 infection was 80% at least 14 days after the first dose and 90% at least 14 days after the second dose. [21]

Moderna also released topline findings from a phase II/III study in teenagers, which revealed that vaccination effectiveness was 100% in populations aged 12 to 17 years old, 14 days after receiving the second dose. [25]

Alpha (B.1.1.7): There was no change in the antibody titres produced against this variant, according to a small laboratory research. Clinical investigations are still needed to establish the impact. [26]

Beta (B.1.351): Antibody titres are decreased by 6.5-fold according to a small laboratory research. Clinical investigations are still needed to establish the impact. [26]

Gamma (P1): Antibody titres are decreased by 2.6-fold according to a small laboratory research. Clinical investigations are still needed to establish the impact. [27]

Delta (B.1.617.2): No evidence yet.

Geriatric Population: In clinical studies, vaccination effectiveness against symptomatic illness 14 days after the second dose was 86.4 % in individuals over 65 years old. [4]

Adverse Effects:

Fever, local discomfort, swelling, soreness, erythema at the injection site, axillary lymphadenopathy, tiredness, headache, myalgia, arthralgia, chills, and nausea/ vomiting were the most prevalent side events in clinical studies after the Moderna COVID-19 vaccination.(Table-3)

JANSSEN VACCINE

Efficacy:

According to findings from a phase III study released on April 21, 2021, vaccine effectiveness against moderate to severe/critical illness was 66.1 % overall 28 days following immunisation. In terms of avoiding severe/critical illness, it was 85.4 % successful. At least 28 days following immunisation, vaccine effectiveness against COVID-19 needing critical care was 100.0 %. [6] (Table1)

Alpha (B.1.1.7): A real-world study published on 30 April 2021 and conducted in the United States when Alpha was the predominant variant circulating found that vaccine effectiveness against SARS-CoV-2 infection was 76.7 % two weeks after vaccination among 1,779 vaccinated individuals matched with 17,744 unvaccinated controls. [28]

Beta (B.1.351): According to Phase III trial results, vaccination effectiveness against moderate to severe/critical disease was 64.0 %, and 81.7 % against severe disease. [6]

Gamma (P1): Phase III trial findings show vaccination effectiveness was 68.1 % against moderate to severe/critical disease and 87.6 % against severe disease due to the gamma variant.[6]

Delta (B.1.617.2): No evidence yet.

Geriatric Population: Phase III trial findings showed that the vaccine had an efficacy of 67.9% 28 days after immunisation in individuals aged 60 years, however vaccine efficacy estimates were greater in persons aged 60 years without comorbidities than in participants aged 60 years with comorbidities. To further understand these possible disparities, more follow-up data is required. [6]

Adverse Effects:

Mild to severe fever episodes occurred after immunisation and disappeared within 1 to 2 days. Injection site discomfort, tiredness, headache, and myalgia were the most common local adverse events. (Table3)

The Janssen vaccination was temporarily halted in the United States from April 13, 2021, to April 23, 2021, due to reports of six thrombocytopenia cases. [39] The CDC had found 28 occurrences of blood clots with insufficient platelets among more than 8.7 million individuals who had been vaccinated as of May 12, 2021.[29] (Table-2)

COVISHIELD VACCINE-

Efficacy:

On March 25, 2021, results from a Phase III study in the United States, Peru, and Chile were published, indicating that when two doses are administered four weeks apart, vaccination effectiveness was 76.4 % in preventing symptomatic illness and 100% against severe COVID-19 infection.[12](Table1)

Alpha (B.1.1.7): Statistics from the United Kingdom (UK) immunisation campaign, published on March 1, 2021, and April 23, 2021, indicated that the vaccine was very effective against the UK variation. Furthermore, despite the fact that there was 9-fold decrease in antibody titres against UK variation, vaccine effectiveness was equal for Alpha and non-Alpha lineages, according to research published in The Lancet on March 30, 2021 (70.4% and 80.5%, respectively). [30]

Beta (B.1.351): According to a modest laboratory research utilising vaccination sera, the number of antibodies that neutralise this variation had decreased by 12.4-fold. According to a research published in The New England Journal of Medicine on March 16, 2021, a two-dose regimen had only a 10.4 % effectiveness in preventing mild to severe COVID-19 infection in young adults. [31]

Gamma (P1): Antibody titres against this variation were decreased by 2.9-fold in a small laboratory study using vaccination sera. [27]

Delta (B.1.617.2): PHE's observational study, which included 1,054 people confirmed to have the Delta variant and was published on May 22, 2021, found that the Oxford/AstraZeneca vaccine had an efficacy of 59.8% against symptomatic disease from the Delta variant two weeks after the second dose, compared to 66.1% against

the Alpha variant. Vaccine effectiveness against the Delta variant was 32.9 % after one dose, compared to 51.4 % against the Alpha version.

PHE issued a revised analysis on 14 June 2021 as a pre-print, which included 14,019 symptomatic patients infected with the Delta strain. According to the study, the delta variant's vaccination effectiveness against hospital admission was 71 % after one dose and 92 % after two doses.

A Scottish research published in The Lancet on June 14, 2021 contained 19,543 instances (7,723 with the Delta variation), with 377 of them requiring hospitalisation (134 with the Delta variant). The vaccination provided 60% (protection against the Delta variant) and 73 %(protection against the Alpha variant) protection.[32]

Geriatric Population: Vaccine effectiveness in people aged 70 years was 56% from 28 - 34 days after getting the vaccine, and improved to 58 % from day 35 onwards, according to data from the UK immunisation programme. The data also reported that one dose of vaccination was around 80% effective at avoiding hospitalization in geriatric population. [24]

The vaccination effectiveness in people aged over 65 years after two doses, four weeks apart, was found to be 85 % in the US trial. [33]

Another observational data from PHE, published in trial on May 10, 2021, showed that one dose of the Covishield vaccine reduced the risk of mortality in people 70 years and older by 55% compared to those who are not vaccinated. First of all, this corresponds to about 80% protection from death in the event of an accident. [24]

Another preprint, released on the same day by PHE, claimed that one dosage of the Covishield vaccine decreased the chance of hospitalization by 73% in people over the age of 80. One dosage decreased the chance of hospitalisation by 84% in those aged 70 to 79.

Adverse Effects:

As of 7 April 2021, the Medicines and Health Products Regulatory Agency (MHRA) reported 79 cases of Thrombocytopenia in the UK, with low platelet counts after 22 million doses of the Covishield vaccine. It is associated with this very rare side effect, but more work is needed.[34]

According to the Joint Committee on Vaccination and Immunization (JCVI) if an alternate vaccination is available, all remaining unvaccinated individuals in phases 1 and 2 of the programme who are 18 to 29 years old and do not have an underlying health condition that puts them at increased risk of severe COVID-19 should be provided it.(Table 2)

SPUTNIK VACCINE

Efficacy:

Results from Phase III trial in the Russia, published on 18 February 2021, reported that the vaccine had an overall efficacy of 91.6% against symptomatic disease 21 days after vaccination and 100% efficacy against moderate or severe COVID-19.[5](Table 1)

Gamma (P1): An Observational study from Brazil/Argentina reported that 99.65% of subjects induced IgG antibodies to COVID-19 on 42nd day after receiving the 2nd dose; 85.5% of subjects induced IgG antibodies to COVID-19 on 14th day after receiving the 1st dose. [35]

Delta (B.1.617.2): The Gamaleya Center study reported that Sputnik vaccine had the most efficacy against the delta variant than any other vaccine. [35]

Geriatric Population: The phase III trial reported that the vaccine had an efficacy of 91.8% of preventing symptomatic COVID-19 in people aged more than 60 year old.[5](Table-4)

Adverse Effects:

The most common adverse effects seen in clinical trials were pain at the injection site, hyperthermia, headache, asthenia and muscle and joint pain and were ranged from mild to moderate and with no severe adverse event.

Update:

The Russian Ministry of Health had validated Sputnik V's efficacy against the Delta variant on July 12, 2021. The vaccine reduced the risk of illness by 6 times and was 83.1%

effective. It was also 94.4% effective in preventing hospitalizations, with a risk decrease of 18 times. According to a non-peer-reviewed study published on August 25, 2021, the Sputnik V vaccination provided 81 percent protection against hospitalisation during the SARS-CoV-2 virus Delta variant surge in Argentina in July-August 2021. [40]

SINOPHARM VACCINE

Efficacy:

When compared to alternative vaccination regimens, the phase 2 study found that 4 µg on day 0 and 21 was linked with the greatest neutralising antibody (GMT). Due to self-limiting grade 3 fever, one grade 3 or higher adverse event was reported. [8](Table 1)

There was no data available on the efficacy against different covid variants.

Geriatric Population: No adequate data available.

Adverse effects:

Adverse responses were reported by 15% of study participants within 7 days of injection. Injection site discomfort was the most prevalent adverse response, followed by fever. All the adverse responses were moderate (grade 1 or 2), transitory, and self-limiting, and no therapy was required.[8]

COVAXIN VACCINE

Efficacy:

The interim reports from phase-3 study showed the vaccine had overall efficacy of 77.8% against symptomatic covid infections.[9]

There was no data available on the efficacy against different covid variants.

Geriatric Population: No adequate data available

Adverse effects:

No serious adverse events were reported in the study. Protective efficacy was not reported.

Update:

COVAXIN had been given an emergency use listing (EUL) by the World Health Organization (WHO) for the prevention of COVID-19 on November 03, 2021. [41]

Discussion

Based on the aspects discussed in this review, we conclude that not all vaccines are equally safe and efficacious. Nevertheless, the risk of severe adverse events or even death was very low. However, questions of efficacy against new variant strains have been raised. The UK variation B.1.1.7 (Alpha variant) has been demonstrated to change the spike protein, which might impair immunological recognition of antibodies generated from current vaccinations. The COVID vaccines Vaxzevria (Oxford – AstraZeneca) and NVX-CoV2373 (Novovax) have been found to be protective against the Alpha variant of the virus, while the latter and Janssen vaccine were protective against the Beta variant (B.1.351). [42] The vaccines Comirnaty and Vaxzevria were found to be 92% and 69% effective respectively against the highly infectious Delta variant (B.1.617.2) of SARS-CoV2, but their protection fades away with time according to a study conducted in the United Kingdom. [43] To evaluate the effectiveness of current vaccinations against mutant versions, more clinical studies are needed and there is an increasing need for inventing vaccines. [44,45]

The vaccinations exhibited limited immunogenicity in older persons over 60(Table-4), but a low risk of adverse reactions. One probable explanation is that elderly population have a lower level of immunity. Many more research on the vaccine's tolerability in the older population are needed. Furthermore, no findings of clinical trials involving juveniles have been reported to far. The majority of studies advocate a two-dose vaccine, although the interval has to be investigated further. In comparison to the other vaccinations evaluated in this study, mRNA vaccines had a greater effectiveness (about 94%).

There is a need for different types of vaccinations for diverse groups, such as babies and children, pregnant women, and immunocompromised people, because most vaccines in development are aimed at the healthy

population, i.e., adults aged 18 to 55.

However, there have been some limitations:

1. There is no proof of the vaccine's long-term efficacy or safety. Most vaccine studies only followed up to 28 days following immunisation due to the necessity of vaccine development. It will need more time to see if neutralising antibodies can be maintained for a long time and if there are any delayed adverse effects following immunisation.

2. Because of variations in the design of numerous clinical studies, it was difficult to compare the benefits and drawbacks of various vaccinations.

3. The published case reports on vaccination adverse effects were short-term, i.e. 1 or 2 weeks after the injection date. As a result, we can't rule out the possibility that these side effects are unrelated to the vaccination. The link between specific adverse effects, like as thrombotic events that occur after taking Pfizer, Moderna, and other vaccinations, has to be investigated further.

In conclusion, the short-term side effects of the vaccine are mild and short-lived. Side effects were more common in younger people (18-55 years) than in older adults. The post-vaccination symptoms often last for one or two days from the injection.

Our findings can assist the public understand the risk of adverse effects based on their age and the type of vaccination they received.

Furthermore, our research backs up the findings of randomised controlled studies that show indications of infection decrease after 12 days and significant protection after 3 weeks.

Conclusion

To summarise the report, adverse effects of the vaccine are minimal and transient with a duration of one or two days. The most affected population were found to be Younger people between 18-55 years age. This Study can help the general public to understand the risk of adverse effects based on their age and type of vaccination. All of the reviewed COVID-19 vaccines have demonstrated promising immunogenicity profile with different degrees of protective effect and a good safety profile. Future studies are needed to evaluate the safety and efficacy of the vaccines among vulnerable population such as immunocompromised patients, geriatrics, paediatrics, pregnant woman and patients with multiple co-morbidities. With the increasing occurrence of new variants of various COVID-19 strains, it is critical to conduct routine efficacy studies. Furthermore, because the clinical trial study population is not very diverse and the vaccines are approved on an emergency basis, long-term postmarketing surveillance becomes an extremely important part of determining efficacy and side effects in different populations.

Conflict of interest: The authors have declared no conflict of interests.

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مراجعة شاملة حول الفعالية والأحداث السلبية المرتبطة بلقاحات COVID-19

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ملخص

لمكافحة COVID-19، أعطت العديد من الوكالات الصحية في جميع أنحاء العالم موافقة طارئة على اللقاحات. ولذلك، فإن الأثر الوقائي الطويل الأجل والآثار الضارة المحتملة للقاحات على المرضى الذين يعانون من نقص المناعة والنساء الحوامل وطب الشيخوخة قد لا يكونان راسخين. وكان الهدف من هذه المراجعة هو تقييم سلامة وفعالية عدد من اللقاحات الأكثر شيوعا المعتمدة في جميع أنحاء العالم. وأجريت مراجعة لتحديد التجارب السريرية التي درست فعالية اللقاحات وتقارير الحالات عن الأحداث الضائرة المحتملة المشتبه في ارتباطها باللقاحات. وكانت قواعد البيانات الإلكترونية التي تم البحث فيها لتحديد الدراسات ذات الصلة هي Science Direct وPubMed/Medline و Scopus و MedRxiv. وشملت المراجعة سبع تجارب معشاة ذات شواهد قيمت فعالية لقاحات كوفيد-19 وتقارير الحالات التي أبلغت عن الأحداث الضائرة للقاحات. وتبين أن فعالية اللقاحات بلغت 94.6٪ للقاح فايزر، و94.1٪ للقاح موديرنا، و66.1٪ للقاح جونسون آند جونسون، و76.4٪ لكوفيشيلد، و91.6٪ لسبوتنيك، و77.8٪ لكوفاكسين. لم يتم الإبلاغ عن أي أحداث سلبية شديدة في الدراسات. وكانت جميع الأحداث الضائرة المبلغ عنها خفيفة ومكتفية ذاتيا ولا تتطلب أي تدخل طبي. أظهرت جميع لقاحات كوفيد-19 ملامح مناعة واعدة، ودرجات مختلفة من الفعالية الوقائية، وملامح سلامة مقبولة. ومع ذلك، هناك حاجة إلى مزيد من البحوث لتقييم الفعالية والسلامة في الفئات السكانية الضعيفة بما في ذلك المرضى الذين يعانون من نقص المناعة والنساء الحوامل والسكان المسنين. تصبح مراقبة ما بعد التسويق على المدى الطويل جزءا مهما جدا من تحديد الفعالية والآثار الجانبية بين مختلف السكان.

الكلمات الدالة: لقاح كوفيد-19، فعالية اللقاح، الآثار الضارة للقاح.

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المجلة الأردنية في العلوم الصيدلانية

تعريف بالمجلة الأردنية في العلوم الصيدلانية

تأسست المجلة الأردنية في العلوم الصيدلانية بقرار لجنة البحث العلمي/ وزارة التعليم العالي والبحث العلمي رقم 367/2/10 تاريخ 2007/1/11 بشأن إصدار "**المجلة الأردنية في العلوم الصيدلانية**" ضمن إصدارات المجلات الأردنية الوطنية، وهي مجلة علمية عالمية متخصصة ومحكمة، وتصدر بدعم من صندوق دعم البحث العلمي والجامعة الأردنية تعنى بنشر البحوث العلمية الأصيلة المقدمة إليها للنشر في كافة مجالات العلوم الصيدلانية والعلوم الأخرى المرتبطة بها. وتصدر عن عمادة البحث العلمي وضمان الجودة في الجامعة الأردنية باسم الجامعات الأردنية كافة، خدمة للمتخصصين والباحثين والمهتمين في هذه المجالات من داخل الأردن وخارجه. وهي مجلة تصدر أربع مرات في العام أعتبارا من 2021، ومواعيد صدورها (آذار وحزيران وأيلول وكانون أول) من كل عام.

وباسمي وباسم أعضاء هيئة التحرير نود أن نشكر الزملاء الذين أسهموا بإرسال أبحاثهم إلى مجلتنا وتمكنا من إخراج العدد الأول. ونأمل من جميع الزملاء بإرسال ملاحظاتهم الإيجابية إلينا لنتمكن من النهوض بمجلتكم بالشكل الذي يليق بها.

وهذه دعوة إلى كافة الزملاء لإرسال اسهاماتهم العلمية من الأبحاث الأصيلة إلى عنوان المجلة.

والله ولي التوفيق

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