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GC-MS Assay to Ethanolic and Aquatic Extract of Mentha spicata L. leaves and Detection Effect of Extract as Antibacterial

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***Abstract*— The analysis and identification of the chemical composition of the aqueous and ethanolic Mentha spicata L. extract by means of gaz chromatography and mass spectroscopy was realized in this paper. In addition, their antimicrobial activity was screened. From the leaf M. spicata, were separated, and determined their composition. The main components of ethanolic extract were Hexadecanoic acid, methyl ester (12.46%), Pentadecanoic acid (15.94%), Octadecanoic acid (6.11%), Methyl stearate (1.84%) and Phytol (28.06%). In Aquatic extract, the main components were Hexadecanoic acid, methyl ester (32.41%), Pentadecanoic acid (26.33%), Methyl stearate (12.33%) and Octadecanoic acid (7.58%). Furthermore, screening of the M. spicata extract for their antimicrobial activities reveals the highest activity of M. spicata alcoholic extract against Staphylococcus aureus, with an inhibition zone 15.8 ± 0.28 in 100% concentration and the highest activity of M. spicata aqueous extract against Pseudomonas aeruginosa, with an inhibition zone 14.0 ± 1.00 in 25% concentration. However, more research on the factors influencing the biosynthesis and bioactivity of chemical composition is needed as aqueous and ethanolic extract have gained important applications in food and pharmaceuticals industry.**

***Keywords*— GC-MS, Antibacterial, Mentha spicata L.**

I. INTRODUCTION

The usage of plant's extracts to treat various diseases has become more widespread, especially due to the increasing of bacterial resistance. This resistance poses a main threat to the health of individuals. Long-term frequent and indiscriminate uses of these medications has led to harmful side effects for individuals [1]. There are many components of plants have not yet known well. These components do not produce directly during photosynthesis but result from subsequent chemical reactions, hence the name "secondary metabolites" [2]. Therefore, the clinical importance of herbal therapeutics (phytotherapy) have received considerable attraction lately. They have considered as a good source for medication because they produce host of bioactive molecules [3]. Medicinal plants contain many bioactive components, like alkaloids, flavonoids, phenolic compounds, steroids, tannins, terpenoids and other secondary metabolites, which have a remarkable effect on parasites and pathogens [4]. These plant's components have unique pharmacological properties, like being affordable, less toxic, with fewer side effects, and less likely to develop resistance [5]. One of these important plants is *M. spicata*. They have been used widely in aromatherapy, pharmacy, cosmetics, and food preservation [6,7]. Moreover, for decades, spearmint (*Mentha Spicata*) belonging to the family Lamiaceae, has been used for its culinary and medicinal properties [8]. In addition, other properties have been discovered in it, such as an effect (antidepressant, anxiolytic) [9,10] anti-inflammatory, antibacterial and antifungal [11,12], hepatoprotective and antioxidant [13]. *M. spicata* is considered one of a perennial herb that has smooth stems, small and green leaves. The plant is about 30–100 cm long. The length and width of the leaves are 5–9 cm and 1.5–3 cm, respectively. Volatile oil, which is present in the tiny, spotted glands on the leaves, gives the plant its aroma and color. The spearmint plant produces flower that are pink and white in color, 2.5–3mm long and broad and arranged in slender spikes [14]. The leaves of *M. spicata* contain some of phytochemical constituents like alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids. All of these components were found in these plants except starch [15]. Misuse and over exploitation of conventional antibiotics has led to the need for development of novel antibacterial medication. Plant's sources with active components have proven to be effective sources for extracting compounds with antibacterial property. The extracts of *M. spicata* have a prominent effect as antibacterial agent on many pathogenic bacterial strains and antioxidant bioactive natural extract [16].

II. MATERIALS AND METHODS

A. Collection and Classification of study stations

Plant samples for this study were collected in Nasiriyah, Thi-Qar Governorate, in southern Iraq, in October 2023 collected leaves of *Mentha spicata*. Dr. Haider Radi, a professor at Thi-Qar University's Faculty of Science, diagnosed the plants. They were thoroughly cleaned of contaminants and processed into a fine powder using an

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electric mill before being stored in sterile glass bottles until use [17].

B. Preparation of plant extract

1) Preparation aqueous Extract

The qualitative screening of chemical compounds was performed by subjecting a mixture of 20 gm of plant powder and 200 ml of distilled water to ultrasonic bath. The solution obtained was passed through a several layers of filter paper Whatman 0.22 and subsequently concentrated at 50°C under decreased pressure using a rotary evaporator. Subsequently, it underwent a drying process at a temperature of 25°C. The extract was ultimately gathered in sterilized glass tubes that are now prepared for utilization [18].

2) Preparation of Ethanol Extract

The qualitative screening of chemical compounds was performed by subjecting a mixture of 20 gm of plant powder and 200 ml of ethanol to ultrasonic bath. The solution obtained was passed through a several layers of filter paper Whatman 0.22. Subsequently, it underwent a drying process at a temperature of 25°C. The extract was ultimately gathered in sterilized glass tubes that are now prepared for utilization [18].

C. Qualitative Analysis of some Phytochemicals

A phytochemical examination was conducted to identify the presence of flavonoids, phenolics, alkaloids, and glycosides. Each analysis, we used10 mg of extracts, following the protocols of [19].

C) GC-MS Analysis of Extracts

The equations involve calculating the percentages of compounds within the extract are an exception to the prescribed Conditions the GC-MS profile was conducted out at GCMS-QP2010 plus device (Shimadzu, Kyoto, Japan) that is equipped with a 5 ms capillary column measuring 30 x 25 mm and a 0.25 μm film thickness, as well as an autoinjector. The carrier gas, helium, has a flow rate of 1.15 milliliters per minute. The 70eV ionized charge system was used to apply mass spectroscopic scanning. After two minutes, the temperature was progressively raised from 80°C to 280°C for five minutes at a rate of 40°C per minute. Splitting mode was applied to the injected samples at 250°C. The National Institute of Standards and Technology (NIST14) and the Wiley 10th/NIST 2014 mass spectral library (W10N14) were the two mass spectral databases used to characterize the separated components using mass spectra and retention times. All analyses were completed at the Industrial Research Directorate of the Ministry of Industry in Baghdad, Iraq [20].

a) Culturing of Samples

For patients suffering from gastrointestinal infections, Imam Hussein Teaching Hospital provided us with bacterial samples. They underwent a VITEK test as well as other biochemical testing to validate the diagnosis of bacteria.

Analytical Profile Index

To identify the isolated bacteria, a fully automated system called VITEK, was used to perform bacterial identification and antibiotic susceptibility testing,

III. RESULTS

Quantitative phytochemical screening test of M. spicata extracts

A. Table 1: Detection of active compounds

Extract of M. spicata	Flavonoid	Phenols	Tannins	Glycoside	Amino acid	Alkaloids
	+	+	+	+	+	+

B. GC-MS analysis of chemical compounds of M.spicata extracs

The chemical analysis of leaves extract of a M. spicata was done using (GCMS) device. Ethanolic Extract of M. spicata were 18 components were identified as shown in Table 1 with percentage of 100%. Aquatic extract had 10 component as shown in (Table 2) with 100%. The main components of ethanolic Extract were Hexadecanoic acid, methyl ester (12.46%), Pentadecanoic acid (15.94%), Octadecanoic acid (6.11%), Methyl stearate (1.84%) and Phytol (28.06%), while in Aquatic Extract, the main components were Hexadecanoic acid, methyl ester (32.41%), Pentadecanoic acid (26.33%), Methyl stearate (12.33%) and Octadecanoic acid (7.58%), while the other chemical compound occupied the other remaining percentage area of M. Spicata extract.

Table 2: Detection of active compounds of aquatic extract of Mentha spicata L.

Aquatic extract of *Mentha spicata* L.				
Peaks	Retention time	Area	Compounds	formula
1	5.978	6.16	Benzoic acid, methyl ester	C8H8O2
2	7.060	1.48	Benzofuran, 4,5,6,7-tetrahydro-3,6-dimethyl-	C10H14O
3	18.372	1.47	3-Methyl-3,5--(cyanoethyl)tetrahydro-4-thiopyranone	C9H13NO2S
4	18.666	32.41	Hexadecanoic acid, methyl ester	C17H34O2
5	19.298	26.33	Pentadecanoic acid	C15H30O2
6	20.977	4.44	9-Octadecenoic acid, methyl ester, (E)-	
7	21.332	12.33	Methyl stearate	C19H38O2
8	21.627	5.95	cis-Vaccenic acid	C18H34O2
9	21.895	7.58	Octadecanoic acid	C18H36O2
10	24.353	1.85	2-[2-Methyl-2-aminoethyl] benzofuran	C12H15NO
Total		100%		

Table 3: Detection of active compounds of ethanolic extract of *Mentha spicata* L.

Ethanoic extract of *Mentha spicata* L.				
Peaks	Retention time	Area	Compounds	formula
1	5.995	1.97	Benzoic acid, methyl ester	C8H8O2
2	17.411	6.42	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)	C10H18
3	17.775	3.50	Phthalic acid, isobutyl 2-isopropoxyphenyl ester	C17H24O4
4	18.009	2.46	Cyclohexanol, 1-ethynyl-	C8H12O
5	18.649	12.46	Hexadecanoic acid, methyl ester	C17H34O2
6	19.290	15.94	Pentadecanoic acid	C15H30O2
7	19.567	1.60	Hexadecanoic acid, ethyl ester	C18H36O2
8	20.969	2.79	11-Octadecenoic acid, methyl ester	C19H36O2
9	21.142	28.06	Phytol	C20H40O
10	21.332	1.84	Methyl stearate	C19H38O2
11	21.583	6.50	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	C18H30O2
12	21.860	6.11	Octadecanoic acid	C18H36O2
13	22.163	0.98	3-(E)-Hexen-2-one, (5S)-5-[(t-butoxycarbonyl-(S)-alanyl)amino]-	C11H19NO3
14	24.318	1.46	9-Octadecenamide, (Z)-	C18H35NO
15	24.500	1.99	Phytol, acetate	C22H42O2
16	26.647	2.09	Phytol, acetate	C22H42O2
17	28.672	2.27	Phytol, acetate	C22H42O2
18	29.183	1.56	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene	C15H26O
Total		100%		

Table 4: Activity of *Mentha* ethanolic extract with different concentrations against bacteria

Con.	Cases No.	*E. cloacae*	*S. aureus*	*P. aeruginosa*	*K. pneumonia*
		M. spicata ethanolic Extract Mean ± S. D			
25%	3	10.0 ± 1.00	12.1 ± 0.28	10.1 ± 1.25	9.83 ± 0.76
50%	3	9.83 ± 0.76	12.5 ± 0.50	10.2 ± 0.28	10.0 ± 1.00
75%	3	0.00 ± 0.00[b]	14.1 ± 0.76	10.6 ± 1.15	11.8 ± 1.60
100%	3	0.00 ± 0.00[b]	15.8 ± 0.28	12.0 ± 0.00	14.7 ± 1.15
p. value		< 0.001	< 0.001	< 0.001	< 0.001
LSD		1.18	0.94	1.63	2.20

Table 5: Activity of Mentha aquatic extract with different concentrations against bacteria

Con.	Cases No.	*E. cloacae*	*S. aureus*	*P. aeruginosa*	*K. pneumonia*
		M. spicata aquatic Extract Mean ± S. D			
25%	3	7.83 ± 0.28	6.33 ± 0.57	14.0 ± 1.00	5.83 ± 0.76
50%	3	10.3 ± 0.57	10.5 ± 0.86	12.6 ± 1.15	8.00 ± 0.50
75%	3	10.3 ± 1.57	12.3 ± 0.57	12.6 ± 0.57	9.83 ± 1.25
100%	3	10.0 ± 0.50	11.6 ± 0.57	10.2 ± 0.28	12.1 ± 0.76
p. value		< 0.001	0.021	0.001	< 0.001
LSD		1.18	1.63	1.24	1.56

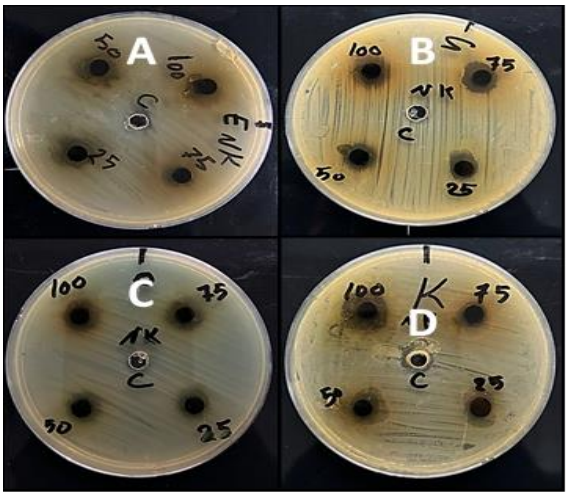

Fig. 1: Activity of Mentha ethanolic extract with different concentrations against growth of (A: E. cloacae, B: S. aureus, C: P. aeruginosa, D: K. pneumonia).

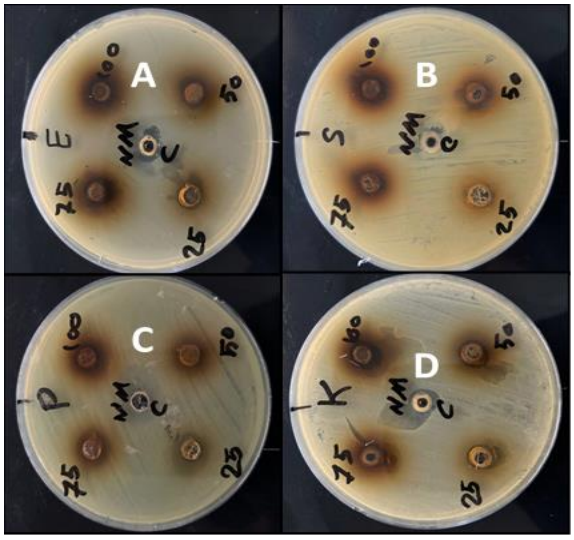

Fig. 2: Activity of Mentha aquatic extract with different concentrations against growth of (A: E. cloacae, B: S. aureus, C: P. aeruginosa, D: K. pneumonia

IV. DISCUSSION

The ability of a substance to either slow down or inhibit the growth of microorganisms such as bacteria, fungus, viruses, and parasites is known as antimicrobial activity. Medicinal plants have been utilized for ages to treat a wide range of ailments after the discovery of using the extraction of plants as antimicrobial chemicals, an important public health concern in recent times has been the rise in antibiotic- and other antimicrobial drug-resistant bacteria [21]. Antimicrobial resistance is one of the top 10 global public health issues affecting humanity, according to the WHO [20]. In this situation, finding novel and potent antibacterial substances are essential. Because medicinal plants produce a wide range of secondary metabolites, many of which have been shown to have antibacterial qualities, and they represent a promising source of these chemicals [22]. Preliminary phytochemical investigation was carried out to know types of phytochemical constituents present in the leaf of Mentha spicata L. According to these results, flavonoid, phenols, tannins, glycoside, amino acid and alkaloids were

found to be present. In the present work, antimicrobial activity of aqueous and ethanolic extracts obtained from the leaf of M. spicata was investigated for different strains of microorganisms such as E. cloacae, S. aureus, P. aeruginosa and K. pneumonia using agar well diffusion method. The antimicrobial activity was observed for the extracts against on four strains of microorganisms. The Mentha ethanolic extract was showed the high activity against S. aureus in 100% concentration, followed against K. pneumonia in 100% concentration. In contrast, the lowest activity in both 50% concentration against *E. cloacae* and 25% concentration against *K. pneumonia*. Furthermore, a non-biological activity against E. cloacae in both 75% and 100% concentration. While the Mentha aquatic extract was showed the high activity against *P. aeruginosa* in 50% and 75% concentrations, followed against S. aureus in 75% concentration. In the other hand, the lowest activity was in 25% concentration against K. pneumonia and 25% concentration against *S. aureus*. From the analysis of extracts by Gas Chromatography with Mass Spectrometer, it was observed that due to the presence of Hexadecanoic acid in the aqueous and ethanolic extracts. This compound has antioxidant, also it has antifungal, antitumor and antibacterial properties and hemolytic activity [23]. Then, chemical pentadecanoic acid possesses antibacterial against multi-drug bacteria[24] and (Methyl stearate). This chemical possesses antibacterial and antioxidant activity as in research by Hagr et al. [25], Antibacterial properties of extracts *M. spicata* were due to the presence of many active chemical compositions according to the results of GC-MS analysis varied in number and percentage [26, 27].

V. CONCLUSION

The current review discussed the chemical constituents of *M.spicata* in Iraq and their pharmacological and therapeutic potentials to enhance their uses in medical practice because of their effectiveness and safety.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

[1] R. A. AlAskary and H. R. Malih, "Effectiveness study of Artemisia *herba-alba* and Borage officinalis leaf extract against bacteria *Staphylococcus aureus*," *Thi-Qar Journal of Science*, Vol. 8, no. 1. Pp. 113-117, 2022.

[2] F. Amarti *et al.*, "Composition chimique, activité antimicrobiennne et antioxydante de l'huile essentielle de *Thymus zygis* du Maroc," *Phytothérapie*, vol. 9, no. 3, pp. 149–157, Apr. 2011,

[3] "Canadian Medicinal Crops," *Google Books*.

[4] H. Archana and V. G. Bose, "Evaluation of phytoconstituents from selected medicinal plants and its synergistic antimicrobial activity," *Chemosphere*, Vol. 8, no. 1. Pp. 113-117, 2022.

[5] S. Wang, X.-Q. Liu, O. Kang, and D. Kwon, "Combination of Sanguisorbigenin and Conventional Antibiotic Therapy for Methicillin-Resistant *Staphylococcus aureus*: Inhibition of Biofilm Formation and Alteration of Cell Membrane Permeability," *International Journal of Molecular Science*, Vol. 23, no. 8. Pp. 4220-4232, 2022.

[6] J. H. C. Nogueira, E. Gonçalez, S. R. Galleti, R. Facanali, M. O. M. Marques, and J. D. Felício, "Ageratum conyzoides essential oil as aflatoxin suppressor of Aspergillus flavus," *International Journal of Food Microbiology*, vol. 137, no. 1, pp. 55–60, Jan. 2010.

[7] L. A. BenSaad, K. H. Kim, C. C. Quah, W. R. Kim, and M. Shahimi, "Anti-inflammatory potential of ellagic acid, gallic acid and punicalagin A&B isolated from Punica granatum," *BMC Complementary and Alternative Medicine*, vol. 17, no. 1, Jan. 2017.

[8] E. Fitsiou *et al.*, "Phytochemical Profile and Evaluation of the Biological Activities of Essential Oils Derived from the Greek Aromatic Plant Species *Ocimum basilicum*, *Mentha spicata*, *Pimpinella anisum* and *Fortunella margarita*," *Molecules*, vol. 21, no. 8, p. 1069, Aug. 2016.

[9] B. Jedi-Behnia, S. Maleki, and E. Mousavi, "The antidepressant-like effect of *Mentha spicata* essential oil in animal models of depression in male mice," *Journal of Advanced Biomedical Sciences*, vol. 7, no. 1, p. 141-49, Aug. 2017.

[10] M. Brahmi *et al.*, "Evaluation of the therapeutic effects of peppermint essential oil on the liver level in developing Wistar rats exposed to lead and manganese," *Carpathian Journal of Food Science & Technology* vol. 11, no. 2, p. 49-55, Apr. 2019.

[11] Barchan, M. Bakkali, A. Arakrak, and A. Laglaoui, "Effet antibactérien et anti-biofilm de trois espèces de *Mentha*: *Mentha spicata*, *Mentha pulegium* et *Mentha piperita*," *Phytothérapie*, vol. 14, no. 2, pp. 88–96, Jun. 2015.

[12] S. Kehili, M. Boukhatem, A. Belkadi, F. Boulaghmen, M. Ferhat, and W. Setzer, "Spearmint (*Mentha spicata* L.) essential oil from tipaza (Algeria): in vivo anti-inflammatory and analgesic activities in experimental animal models, *Acta Pharmaceutica Hungarica*, vol. 1, no. 10, pp. 15–26, Jun. 2020.

[13] Chrysargyris, P. Xylia, G. Botsaris, and N. Tzortzakis, "Antioxidant and antibacterial activities, mineral and essential oil composition of spearmint (*Mentha spicata* L.) affected by the potassium levels," *Industrial Crops and Products*, vol. 1, no. 103, pp. 202–212, Jun. 2017.

[14] F. Anwar, A. Abbas, T. Mehmood, A. Gilani, and N. Rehman, "Mentha: A genus rich in vital nutra-pharmaceuticals—A review," *Phytotherapy Research*, vol. 33, no. 10, pp. 2548–2570, Jul. 2019.

[15] M. Snoussi, E. Noumi, N. Trabelsi, G. Flamini, A. Papetti, and V. De Feo, "*Mentha spicata* Essential Oil: Chemical Composition, Antioxidant and Antibacterial Activities against Planktonic and Biofilm Cultures of Vibrio spp. Strains," *Molecules*, vol. 20, no. 8, pp. 14402–14424, Aug. 2015.

[16] T. Mata, C. Proença, A. R. Ferreira, M. L. M. Serralheiro, J. M. F. Nogueira, and M. E. M. Araújo, "Antioxidant and ant acetylcholinesterase activities of

five plants used as Portuguese food spices," *Food Chemistry*, vol. 103, no. 3, pp. 778–786, Jan. 2007.
[17] N. N. Hussein and H. R. Malih, "Inhibitory Effect of *Tamarix aucheriana* aqueous and Alcoholic Extracts against *Klebsiella Pneumonia* Which Isolated from Patients in Nasiriya City, Southern Iraq," *Annals of the Romanian Society for Cell Biology*, vol. 25, no. 6, pp. 12220–5, Jun. 18, 2021.
[18] Y. Liu *et al.*, "Response surface optimization of ultrasound-assisted enzymatic extraction polysaccharides from *Lycium barbarum*," *Carbohydrate Polymers*, vol. 110, pp. 278–284, Sep. 2014.
[19] B. Pradhan, S. Patra, S. R. Dash, R. Nayak, C. Behera, and M. Jena, "Evaluation of the anti-bacterial activity of methanolic extract of Chlorella vulgaris Beyerinck [Beijerinck] with special reference to antioxidant modulation," *Future Journal of Pharmaceutical Sciences*, vol. 7, no. 1, Jan. 2021, doi: 10.1186/s43094-020-00172-5.
[20] J. B. Harborne, *Phytochemical Methods Book*. 1984. doi: 10.1007/978-94-009-5570-7.
[21] E. Mezal, Z. K. Hanan, and M.Saleh, "Determination of antibiotic resistance of *Salmonella* enterica isolated from children with diarrhea in Thi Qar Governorate during 2015, thesis"" 2016.
[22] "WHO model list of essential medicines, 20th list (March 2017, amended August 2017)," 2017. https://iris.who.int/handle/10665/273826
[23] C. Jain, S. Khatana, and R,Vijayvergia, "Bioactivity of secondary metabolites of various plants: A review," Int. J. Pharm. Sci. Res., vol. 10, no. 2, pp. 494–504, 2019.
[24] T. Tyagi and M. Agarwal, "Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms," *www.phytojournal.com*, vol. 6, no. 1, pp. 195–206, vol. 10, no. 1, pp. 17–26, 2017.
[25] T. Mujtaba, S. Hassan, A. Hassan, M. Wajdi, A. Hafeez, K. Shereen, K. Hussain, A. Ahmed, H. Furqan, N. Tabassum, and M. A. Akbar, "Electron ionization gas chromatography-mass spectrometry (EI-GC-MS) analysis of extracted oil from *Tribulus terrestris* seeds," *Pakistan Journal of Pharmaceutical Sciences*, vol. 33, pp. 2633–2641, 2020.
[26] P. Das *et al.*, "Antibacterial activity and molecular docking study of Coptis teeta," Pakistan Journal of Pharmaceutical Sciences, *Herba Polonica*, vol. 69, No. 2, pp. 1-8, 30 May, 2023.
[27] T. E. Hagr and I. Adam, "Phytochemical Analysis, Antibacterial and antioxidant Activities of Essential Oil from *Hibiscus sabdariffa (L)* Seeds, (Sudanese Karkadi)," *Pakistan Journal of Pharmaceutical Sciences, Herba Polonica*, vol. 3, No. 3, pp. 194-201, 1 Jul, 2020.