Pakistan J. Zool., pp 1-10, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20240309051129

Evaluating the Chemical Composition and Antibacterial and Antioxidant Effects of the Essential Oil of *Melissa officinalis* L.

Salman Heidarian[1]*, Mehrdad Ataie Kachoie[1,2]*, Sadegh Mousavi-Fard[1,3] and Fariborz Moattar[2,4]

[1]*Department of Medicinal Plants, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran*
[2]*Medicinal Plants Processing Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran*
[3]*Department of Horticultural Science, Faculty of Agriculture, Shahrekord University, Shahrekord P.O. Box 115, Iran*
[4]*Department of Pharmacology, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran*

Article Information
Received 09 March 2024
Revised 06 April 2024
Accepted 18 April 2024
Available online 12 September 2024 (early access)

Authors' Contribution
SH, SM-F and FM: carried out computational and experimental jobs. MAK: conceived of the presented study. MAK: Wrote the manuscript and discussed the results and contributed to the final manuscript.

Key words
Medicinal plant, *Melissa officinalis*, Essential oil, Antimicrobial, Molecular docking

ABSTRACT

The present study aimed to assess the antimicrobial effects of *Melissa officinalis* L. essential oil against pathogenic bacteria. Numerical data collected from the experiment were statistically analyzed using SPSS/21.0 software (SPSS Inc., Chicago, IL). Thirty-four (99.95%) chemical components were identified in the *M. officinalis* essential oil, the dominant compounds being Geranial (27.92%), Neral (22.2%), (Z)-Caryophyllene (11.77%), (E)-Caryophyllene (3.18%), and Caryophyllene oxide (3.85%). Dose-dependent antimicrobial effects were observed, with the highest diameters of growth inhibition zones being recorded against 4 mg ml^{-1} *M. officinalis* essential oil for *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Acinetobacter baumannii*. Furthermore, the lowest minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were obtained for the *M. officinalis* essential oil against *P. aeruginosa* and *E. coli* bacteria. Molecular docking revealed that the highest effect of Caryophyllene, Caryophyllene oxide, Geranial and Neral compounds against D-transpeptidase MrdA of *E. coli* was observed for Caryophyllene oxide, with a high energy binding of -6.78 kJ mol^{-1}. Taking into account the high diameter of the growth inhibition zone and low MIC and MBC levels of 4 mg ml^{-1} *M. officinalis* essential oil may serve as an economical source of antimicrobials.

INTRODUCTION

Using of herbs with medicinal properties has been in various medical contexts from a long time ago (Widoyo *et al*., 2023; Ebrahimi *et al*., 2022; Bagherzadeh- Lakani *et al*., 2024; Kiani and Akbary, 2023). This is mainly because of the more availability, affordable, and low side effect of these natural sources. So, it consider some of them as antibacterial, antiviral, anticancer and antioxidant agents (Basati *et al*., 2019; Ghamari *et al*., 2017). For example, the nutraceutical sector with ability of creating new phyto complexes from food and plant-derived substances have beneficial effects (Abbasi *et al*., 2014; Aidy *et al*., 2020). That's why, we focus on a plant (the Lamiaceae family) with antibacterial and anticancer in this study.

One of the perennial subshrub of this family is *Melissa officinalis* L. (lemon balm) that is endemic to Europe and Central Asia, and is widely cultivated in Romania, Spain, Bulgaria, and Turkey (Jamal-Omidi *et al*., 2018). *M. officinalis* plant is a multi-year herbaceous plant with many branches, 30-80 cm high, and wide, egg-shaped leaves, 3-6 cm long, and oppositely dark green with an uneven surface and numerous ridges, flowers in The upper part of the plant and formed in the corner of the leaves, the buds are usually pale, which turn into white or purple flowers after opening. Hazelnut fruit has 4 parts and its length is 1-1.5 mm. Flowers appear in mid-summer. The

* Corresponding author: heidarians@ymail.com, drataie@iaushk.ac.ir
0030-9923/2024/0001-0001 $ 9.00/0

seed is dark and shiny (black). The small root is cylindrical, hard and relatively branched. There are about 1500 seeds in one gram and the weight of 1000 seeds is 0.6-0.7 grams (Marongiu *et al*., 2004; Rădulescu*et al*., 2021).

In fact, *M. officinalis* L. plant has three subspecies with commercial value such as *Melissa officinalis*, *Inodona*, and *Altissima* (Marongiu *et al*., 2004; Rădulescu*et al*., 2021). Among them, *officinalis* has been extensively cultivated for its characteristic lemon-scented essential oil due to its digestive, antispasmodic, antibacterial, antiparasitic, antioxidant, and antiviral activity (Mimica-Dukic *et al*., 2004; Ambreen *et al*., 2022; Lin *et al*., 2012). Leaves of *M. officinalis* contain 0.05–0.15% essential oils in fresh material and 0.1–0.45% essential oils in dried material, respectively (Ebadollahi *et al*., 2016) that it has mild abdominal disorders, biliary dyskinesia (Shakeri *et al*. 2016) and inhibitor the growth of pathogens (Noshad *et al*., 2018) properties (Shakeri *et al*. 2016). Besides, *Melissa* essential oils commonly used in the food and pharmaceutical industries (Miraj *et al*., 2017), and high-priority pathogens (Tacconelli, 2017).

Many research groups use from computational study for drug design due to high cost and time cunsuming of experimental methods (Darvishi, 2016; Negahdari *et al*., 2019). One of structure based drug design (SBDD) method is molecular docking. In this experimental study, chemical compounds, antibacterial and antioxidant activities of *M. officinalis* essential oil and their interaction with target proteins were investigated.

MATERIALS AND METHODS

Plant material and essential oils preparation

Aerial parts of *M. officinalis* plants were collected from Shahrekord city, Iran in 2020, and identifed by the Department of Medicinal and Aromatic Plants, Islamic Azad University, Shahrekord Branch. A voucher specimen (Herbarium No. 1966) was deposited in the Agricultural Research and Training Center and Natural Resources of Chaharmahal and Bakhtiari Province. The plant material was air-dried at an ambient temperature of 20±2°C. The essential oils was obtained through water distillation (UK Pharmacopoeia) using Cloninger apparatus. The 50 g of the dried aerial parts of *M. officinalis* plant were weighed and the essential oils were extracted for 3 h. The obtained essential oils was dehydrated using sodium sulfate and was stored at 4°C in dark glass vessels until further analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

The obtained essential oils, after being concentrated using a rotary evaporator device, stored at -18°C for 48 h. The liquid phase of the essential oils. isolated using filter paper and blended with an equal amount of hexane. Then, mixture was placed on a shaker at 186 rpm for 1 h before being left to settle for 15 min, producing two separate phases. The phase was containing the *M. officinalis* essential oils used for injection into a GC-MS device (Hewlett Packard, HP-6890, USA). The device was equipped with an HP-1MS (methyl silicon-cross link) column (60 m length, 0.2 mm diameter, and 0.25 µm film thickness). The carrier gas, helium of 99.99% purity, was kept at a constant flow rate of 1 ml min^{-1}. In the GC analysis, the column temperature was adjusted to 35°C for 5 min and then increased to 160°C at a rate of 7°C per min (residence time 10 min) before further increasing to 230°C at a rate of 15°C per min (residence time 10 min). The injection ratio was 1:20 (split). The injection chamber and transmission line temperatures were set to 250°C. The MS device (Hewlett Packard, HP-5970, USA) operated at an ionization energy of 70 eV, with a full sweep status of 20–550 m z^{-1}. The ionization chamber and mass decomposer temperatures were set to 230°C and 150°C, respectively. Alkanes identified relative to commercial standards, while other compounds were tentatively identified by comparing their MS spectra with those of commercially available libraries (Wiley Registry of Mass Spectral Data, sixth ed.; NIST/EPA/NIH Mass Spectral Library 1.5a). Molecular weights were determined either by identifying the $(M)^+$ peaks or their $(M-15)^+$ fragments. Acid $(RCO_2H_2)^+$ fragments were used to identify homologous isomers of wax esters eluting in one peak, whose intensity was used to calculate the composition of the respective isomers. In some cases, the corresponding alcohol fragments $(R'-1)^+$ were also detected in the mass spectrum (Etame *et al*., 2018). The relative amounts of the identified compounds were calculated by integrating the peak area and expressed as a percentage of the total area of all recognized peaks in the TIC chromatogram. The values presented in this article are the means of three independent wax analyses.

Evaluation of antibacterial activity

Four pathogenic bacterial strains, namely *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 9144), *Acinetobacter baumannii* (ATCC 19606), and *Pseudomonas aeruginosa* (ATCC 25922) were sourced from the Microbiology Research Center of the Pasteur Institute of Iran, in Tehran, Iran. The pure cultures were cultured separately in tryptic soy broth (Merck, Germany) and incubated at 37°C for 24 h for regeneration.

Agar disk diffusion assay

The antimicrobial activity of *M. officinalis* essential oils was evaluated using the disk diffusion method. After overnight incubation, the bacterial concentration

reached 1×10^6 CFU ml^{-1}. The bacteria were then cultured in Müller-Hinton agar medium. Blank discs of 6 mm diameter were placed on the medium, and 1000 µL of *M. officinalis* essential oils with concentrations of 0.5, 1, 2, and 4 mg ml^{-1} were applied to the discs. For comparison, ceftazidime (30 µg), imipenem (10 µg), gentamicin (10 µg), vancomycin (30 µg), penicillin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), erythromycin (15 µg), ampicillin (10 µg), and azithromycin (15 µg) (Mast, UK) antibiotics were also tested. Each disc was placed at regular intervals on the plates containing the bacteria and the plates were then incubated for 24 h at 37 °C. The diameter of the growth inhibition zones around the discs was measured in millimeters (Khameneh *et al*., 2019).

Minimum inhibitory concentrations (MIC) and minimum bacterial concentrations (MBC)

Fresh cultures of bacteria were obtained in a Mueller–Hinton broth medium (Merck, Germany) to create a turbidity of 0.5 McFarland. This turbidity was then diluted to a ratio of 1 to 100, resulting in a concentration of 1×106 CFU ml^{-1}. Subsequently, 8 µL of different dilutions of *M. officinalis* essential oil containing 2 µL of the bacterial suspension were added to polystyrene plates. Additionally, wells containing 4 µL of broth medium served as negative control, while wells containing culture medium and bacteria served as positive control. Wells composed of 2 µL of the medium and 1 µL of each dilution were also used as a control for turbidity assessment. The tests were performed in triplicates. The plates were then covered and incubated at 4 °C for 4 h. After incubation, the turbidity was read at 630 nm using an ELISA reader (Statfax 2100, USA). The lowest concentration of *M. officinalis* essential oils that reduced the turbidity by 90% compared to the control group was considered the MIC, while the lowest concentration of *M. officinalis* essential oils that caused complete turbidity removal was considered the MBC. For comparison, the MIC and MBC of antibiotics were also determined (Khalili *et al*., 2018). Ceftazidime, imipenem, gentamicin, vancomycin, penicillin, ciprofloxacin, tetracycline, erythromycin, ampicillin, and azithromycin (Mast, UK) antibiotics were prepared in powder form and then serial concentrations of 0.5, 1, 2, and 4 mg ml^{-1} were created using dilution in sterile water. A negative control experiment was conducted using only sterile water.

Molecular docking

Protein preparation

Three-dimensional structure of the D-transpeptidase enzyme from *E. coli* was obtained from the protein data bank (PDB) repository with the identification code 6G9P. The crystallographic water molecules were excluded from the protein structure. To ensure accuracy, the chemistry of the protein was modified by adding missing hydrogen atoms and correcting any crystallographic disorder and unfilled valence atoms utilizing the alternate conformations and valence monitor options. This particular structure was chosen due to its high structural similarity to that of *Staphylococcus aureus* (96%) and *Acinetobacter baumannii* (81.52%), as determined by the PDB (Fig. 2).

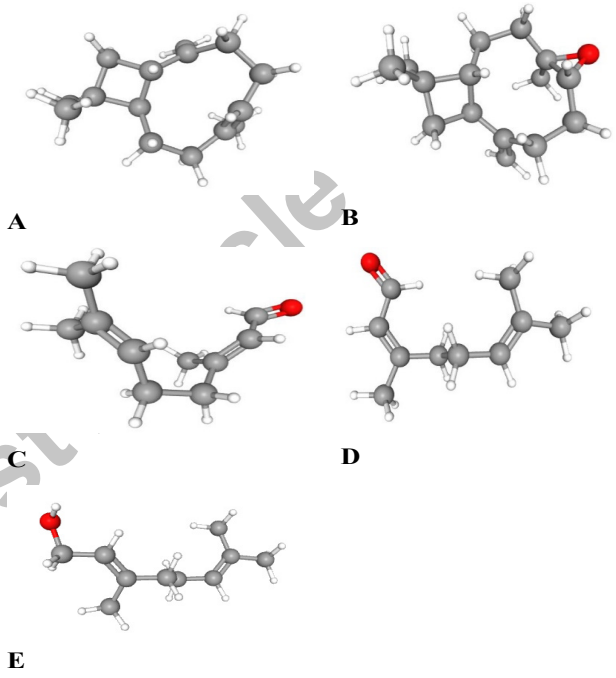

Fig. 1. Chemical structure of selected molecules of *M. officinalis* essential oils Caryophyllene (A), Caryophyllene oxide (B), Geranial (C) and Neral (D) Geraniol (E).

Ligand preparation

Three-dimensional structures of Caryophyllene, Caryophyllene oxide, Geranial, Geraniol, and Neral compounds of the *M. officinalis* essential oils were retrieved from scientific databases such as PubMed and OpenBabel. Subsequently, hydrogen bonds were added and energy optimization of the ligand was performed by using the MM+ force field. The optimized structures of the compounds are depicted in Figure 1. This force field is a widely used and well-validated force field in computationalstudies that applies classical mechanical potential to system. It provides a reliable approximation of the interaction energies of molecules in terms of electrostatic, van der Waals, and other non-bonded components. Therefore, the use of this force field is suitable for accurately describing three-dimensional structure of the *M. officinalis* essential oils compound in this study (Tsai et al., 2020).

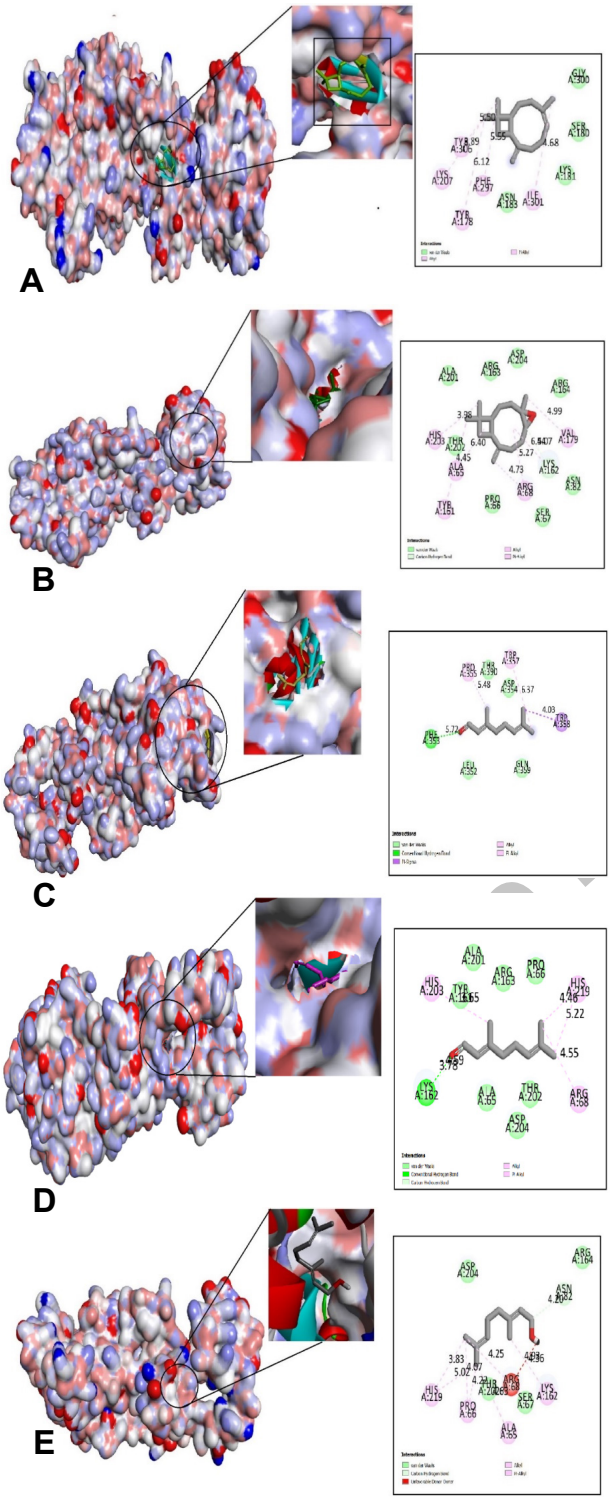

Fig. 2. Nonbonding interactions of best selected phytochemicals Caryophyllene (A), Caryophyllene oxide (B), Geranial (C) and Neral (D) Geraniol (E) of *M. officinalis* with D-transpeptidase of *E. coli*.

Table I. Chemical compositions of *M. officinalis* essential oils by gas chromatography-mass spectrometry analysis.

No.	Components	RI	Area (%)
1	1-Octen-3-ol	968	0.18
2	6-methyle-5-hepten-2-one	989	0.4
3	Linalool	1096	0.73
4	cis, cis- Photocitral A	1137	0.29
5	Exo-Isocitral	1142	0.15
6	cis-β-Terpineol	1147	0.42
7	Citronellal	1150	0.62
8	(Z)-Isocitral	1161	0.84
9	Neo-iso- Isopulegol	1172	0.37
10	(E)-Isocitral	1180	1.42
11	p-Cymen-8-ol	1185	0.49
12	Decanal	1206	0.99
13	Nerol	1228	5.76
14	Neral	1243	22.2
15	Geraniol	1255	6.81
16	Methyl citronellate	1258	0.48
17	Geranial	1274	27.92
18	Thymol	1291	1
19	Methyl geranate	1323	0.97
20	α-Copaene	1376	0.31
21	Geranyl acetate	1383	4.71
22	(Z)-Caryophyllene	1409	11.77
23	(E)-Caryophyllene	1421	3.18
24	α-Humulene	1455	0.9
25	Aromadendrene	1462	0.17
26	γ-Cadinene	1524	0.55
27	α-Calacorene	1554	0.19
28	Caryophyllene oxide	1586	3.85
29	Humulene epoxide II	1612	0.21
30	Caryophylla-4(12),8(13)-dien-5α-ol	1639	0.15
31	epi-α-Muurolol	1644	0.5
32	α-Cadinol	1657	0.93
33	Oplopanone	1673	0.25
34	Hexadecanoic acid	1958	0.24
		Total	99.95

Docking procedure

In this study, Auto Dock (V. 4.2) was utilized to perform docking experiments (Pirbalouti et al., 2019) The parameters of the Lamarckian Genetic Algorithm (LGA) applied in the analysis included 30 independent runs, a population size of 150, a maximum number of 25 million energy evaluations, 27,000 generations, a mutation rate of 0.02, and a crossover rate of 0.8. Blind docking was employed and the grid size was adjusted to encompass the entire enzyme. The RMS cluster tolerance

was set to 2.0. Autodock tools was utilized to compile, collect, and extract the generated conformations. The first and last conformation from a 100-ranked set of each complex were then analyzed using discover studio 2016. This experimental approach allowed us to explore and analyze the interactions between the compounds and the target enzyme in a comprehensive manner. Moreover, the parameters used for the docking experiments were optimized to allow for an efficient exploration of the conformational space of the enzyme-ligand complexes (Szliszka et al., 2009).

Statistical analysis

Numerical data collected from experiment were statistically analyzed using SPSS/21.0 software (SPSS Inc., Chicago, IL). The results were evaluated using a completely randomized design with Minitab 19 software. Qualitative data obtained from the tests were analyzed using the Chi-square test and Fisher's exact two-tailed test. The significance level was determined as p-value less than 0.05.

RESULTS

In this study, antimicrobial effects of *M. officinalis* essential oils were assessed against pathogenic bacteria. First, chemical composition of *M. officinalis* essential oils was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Then, the antimicrobial effects were evaluated using disk diffusion, the MIC and MBC evaluation, and molecular docking. Table I presents the phytochemical compounds identified in the essential oils. Altogether, 34 (99.5%) chemical components were identified. Geranial (27.92%, $C_{10}H_{16}O$), Neral (22.2%, $C_{10}H_{16}O$), (Z)-Caryophyllene (11.77%, $C_{15}H_{24}$), (E)-Caryophyllene (3.18%, $C_{15}H_{24}$), and Caryophyllene oxide (3.85%, $C_{15}H_{24}O$) were the most abundant compounds in the essential oils. Citral, a mixture of Neral and Geranial, made up 50.12% of the essential oils compounds.

Diameters of growth inhibition zones of examined bacteria in response to the *M. officinalis* essential oils and antibiotic discs are given in Table II. Average diameter of tested bacteria's growth inhibition zone increased with increasing concentrations of *M. officinalis* essential oils. The greatest diameter of the growth inhibition zone was observed against the 4 mg ml^{-1} concentration of *M. officinalis* essential oils for all of the examined bacteria. The largest diameters of the growth inhibition zones of *P. aeruginosa*, *E. coli*, *S. aureus*, and *A. baumannii* bacteria were found against 4 mg ml^{-1} of *M. officinalis* essential oils (14.21±0.74 mm), 4 mg ml^{-1} of *M. officinalis* essential oils (13.38±0.36 mm), imipenem (14.61±0.29 mm), and imipenem (10.01±0.31 mm), respectively. Statistically significant differences were observed in the diameter of the growth inhibition zone of the examined bacteria between some different concentrations of *M. officinalis* essential oils and antibiotic agents ($P < 0.05$).

Table II. The growth inhibition zone diameter of examined bacteria toward *M.* officinalis essential oils and antibiotic discs.

Essential oil and antibiotics/ concentrations		Diameter of the growth inhibition zone (mm)			
		P. aeruginosa	***E. coli***	***S. aureus***	***A. baumannii***
M. officinalis essential oils	4	14.21±0.74 ^{a***}	13.38±0.36 ^{a}	10.72±0.10 $_{b}$	9.19±0.14 $_{a}$
	2	13.70±1.02 $_{a}$	12.99±0.18 $_{a}$	9.88±0.15 $_{c}$	8.20±0.29 $_{b}$
	1	12.03±0.55 ^{b}	11.01±0.29 ^{b}	9.22±0.41 ^{c}	7.95±0.19 ^{bc}
	0.5	11.82±0.33 ^{b}	10.17±0.24 ^{b}	8.27±0.33 ^{d}	7.30±0.22 ^{c}
Antibiotics	Cef**	11.51±0.64 $_{b}$	12.17±0.35 $_{a}$	13.42±0.93 $_{a}$	9.13±0.29 $_{a}$
	Imp	12.15±0.50 ^{b}	12.93±0.48 ^{a}	14.61±0.29 ^{a}	10.01±0.31 ^{a}
	G	10.41±0.60 ^{b}	10.53±0.28 ^{b}	11.14±0.22 ^{b}	7.20±0.35 ^{c}
	V	11.90±0.75 ^{b}	12.86±0.63 ^{a}	13.97±0.49 ^{a}	9.92±0.71 ^{a}
	P	10.65±0.39 ^{b}	11.51±0.40 ^{b}	12.08±0.27 ^{b}	8.23±0.45 ^{b}
	Cip	10.96±0.27 ^{b}	11.95±0.57 ^{ab}	12.58±0.39 ^{b}	8.77±0.41 ^{b}
	Tet	10.37±0.56 ^{b}	10.60±0.14 ^{b}	11.02±0.18 ^{b}	7.07±0.27 ^{c}
	Er	10.89±0.44 ^{b}	11.72±0.48 ^{ab}	12.39±0.26 ^{b}	8.61±0.53 ^{b}
	Am	10.77±0.42 ^{b}	11.25±0.56 ^{b}	11.83±0.36 ^{b}	8.05±0.20 ^{b}
	Az	11.30±0.55 ^{b}	12.26±0.18 ^{a}	13.22±0.46 ^{a}	9.19±0.14 ^{a}

Dissimilar letters in each column show statistically significant differences about $P < 0.05$. Cef, Ceftazidime; Imp, Imipenem; G, Gentamicin; V, Vancomycin; P, Penicillin; Cip, Ciprofloxacin; Tet, Tetracycline; Er, Erythromycin; Am, Ampicillin; Az, Azithromycin.

Table III illustrates the MIC and MBC values of *M. officinalis* essential oils and antibiotic agents on the tested bacteria. Results indicate that the *M. officinalis* essential oils yielded the lowest MIC and MBC values against *P. aeruginosa* and *E. coli* bacteria, which is significantly lower than the MIC and MBC values of ampicillin, tetracycline, and gentamicin antibiotic agents. This suggests that *M. officinalis* essential oils is more effective than antibiotic agents in inhibiting the growth of *P. aeruginosa* and *E. coli* bacteria. In contrast, ampicillin, tetracycline, and gentamicin antibiotic agents exhibited the highest MIC and MBC values, indicating that their efficacy in controlling the growth of these bacterial species is relatively low.

Table III. The MIC and MBC indexes of *M. officinalis* essential oils and antibiotic agents.

Essential oil/ anti-biotics	*P. aeruginosa*		*E. coli*		*S. aureus*		*A. baumannii*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Essential oil	0.5	1	0.5	1	1	2	1	2
Cef*	1	2	1	2	0.5	1	2	4
Imp	0.5	1	0.5	1	0.5	1	0.5	1
G	2	4	2	4	2	4	ND	ND
V	1	2	0.5	2	0.5	1	2	4
P	2	4	1	2	1	2	4	ND
Cip	2	4	1	2	1	2	4	ND
Tet	2	4	2	4	2	4	ND	ND
Er	2	4	2	4	1	2	4	ND
Am	2	4	2	4	2	4	-	ND
Az	1	2	50	100	1	2	4	ND

Molecular docking analysis of the essential oil of *M. officinalis* revealed that out of the four compounds tested, Caryophyllene oxide had the strongest affinity for the D-transpeptidase enzyme of *E. coli* bacteria. This was evidenced by the presence of multiple hydrogen bonds and hydrophobic interactions between two molecules. The high affinity of Caryophyllene oxide for the D-transpeptidase enzyme suggests that it has the potential to inhibit or disrupt the enzyme's activity, which could in turn inhibit the growth of *E. coli* bacteria.

Electrostatic energy and van der Waals energies, as well as the lowest binding energy from docking between Caryophyllene, Caryophyllene oxide, Geranial, and Neral and D-transpeptidase of *E. coli* are shown in Table IV. Geranial had the lowest binding energy against D-transpeptidase *E. coli* at -4.79 kJ mol^{-1}. Caryophyllene oxide had the highest binding energy against *A. baumannii* at -6.78. Therefore, Caryophyllene oxide from *M. officinalis* essential oil demonstrated the strongest antimicrobial effects against *E. coli*. Additionally, the electrostatic energy was lower than van der Waals in this connection. In this table, results of the binding of the evaluated compounds with the receptor are shown. A hydrogen bond was observed between geranial and the amino acid phenylalanine 353 (Phe: 353), as well as between neral and the amino acid lysine 162 (Lys:162) with the D-transpeptidase.

DISCUSSION

Plant-derived antimicrobial agents are well-known for their ability to inhibit the growth of microorganisms, including bacteria, fungi and parasites. Inhibition of protein synthesis, interference with cell wall synthesis, inhibition of metabolic pathways, interference with nucleic acid synthesis and disruption of cell cytoplasmic membrane are the main mechanisms of their antimicrobial effects (Abdellatif et al., 2014). Furthermore, numerous studies have reported that geranial, neral, citronellal, and caryophyllene oxide were the predominant chemical

Table IV. Electrostatic energy and van der Waals energies and the lowest binding energy from docking Caryophyllene, Caryophyllene oxide, Geranial and Neral (D) of *M. officinalis* with D-transpeptidase of *E. coli*.

Molecule	Binding energy	Ki (μM)	E vdw + H-bond + Desolvation energy	Electrostatic energy	Hydrogen bonds	Hydrophobic bonds
Caryophyllene	-6.63	13.82	-6.63	+0.00	-	Ser(A):180, Lys(A):181, Asn(A):183, Gly(A):300
Caryophyllene oxide	-6.78	10.72	-6.74	-0.04	-	Pro(A):66, Ser(A):67, Asn(A):82, Lys(A):162, Arg(A):164, Asp(A):204, Arg(A):163, Ala(A):201
Geranial	-4.79	308.38	-5.99	+0.00	Phe(A):353	Leu(A):352, Asp(A):354, Gln(A):359, Thr(A):390
Neral	-4.89	261.99	-6.08	+0.00	Lys(A):162	Ala(A):65, Pro(A):66, Thr(A):161, Ala(A):201, Thr(A):202, Asp(A):204
Geraniol	-4.73	340.00	-6.04	-0.19	-	Asp(A):204, Arg(A):164, Asn(A):82, Ser(A):67, Thr(A):202

components in *M. officinalis* essential oil (Jalal et al., 2015; Hamad et al., 2021; Abers et al., 2021) with most frequently identified chemical components. Variations in the chemical composition of essential oil can be attributed to environmental and genetic factors, post-harvest processing, geographical area, climate, season of sampling, plant part, plant phenological stage, and method of chemical components identification (Klūga et al., 2017). In addition, the geographical area, climate, collection season, part of the plant (including aerial parts, stems, and roots), phenological stage of the plant at the time of collection, and method of component identification, variations in the chemical composition profile of medicinal and aromatic plants have been observed. This can be attributed to the aforementioned factors, as the environment and conditions under which the plants are grown can cause differences in the chemical composition of the plants. Furthermore, the method of component identification can affect the chemical composition profile, as different techniques may yield different results.

Our study demonstrated the potent antimicrobial properties of *M. officinalis* essential oil against two common clinical bacterial strains, namely *P. aeruginosa* and *E. coli*. By administering 4 mg ml^{-1} of *M. officinalis* essential oil, the highest antimicrobial effect was observed. The growth inhibition zone of *P. aeruginosa* treated with *M. officinalis* essential oil was larger than all antibiotic agents examined ($P < 0.05$). Similarly, the growth inhibition zone of *E. coli* treated with 4 mg/mL *M. officinalis* essential oil was significantly larger than that of the gentamicin, penicillin, tetracycline, and ampicillin antibiotic agents ($P < 0.05$).

The antimicrobial effects of *M. officinalis* essential oil can be attributed to the presence of chemical components such as citronellal, caryophyllene oxide, geranial, and neral, the antimicrobial effects of which have been reported in previous surveys (Ehsani *et al*., 2017; Jafarzadeh *et al*., 2020). Contrary to other findings indicating higher susceptibility of Gram-positive bacteria (e.g., *S. aureus*) to natural essential oils than gram-negative bacteria (e.g., *E. coli* and *P. aeruginosa*) (Yu *et al*., 2022; Arzhang *et al*., 2015), our results showed that *M. officinalis* essential oil had higher antimicrobial effects against *P. aeruginosa* and *E. coli*. Similar findings were obtained (Arzhang *et al*., 2015; Jafari-Sales *et al*., 2020).

Collected *M. officinalis* essential oil from Algeria was found to have remarkable antimicrobial effects against gram-negative bacteria such as *B. subtilis*, *P. aeruginosa*, *E. coli*, *K. pneumonia*, and *S. enterica*, as determined by the disk diffusion technique. This suggests that *M. officinalis* essential oil may be a promising alternative treatment for infections caused by gram-negative bacteria. Furthermore, essential oils have been found to have fewer side effects than traditional antibiotics (Islam *et al*., 2019) further reinforcing the potential of *M. officinalis* essential oil as a viable treatment option for gram-negative bacterial infections. Our results showed that the compound had a high negative binding energy, indicating its strong antibacterial activity against *E. coli*. This finding was further confirmed by disk diffusion and MIC and MBC analyses; these results are in good agreement with other report (Dorman and Deans, 2000). Antibiotic, anti-microbial, antioxidant properties of many medicinal plants due to the presence of phenolic compounds, flavonoids, flavonoids, tannins, anthocyanins and plant antioxidants (Shahsavari *et al*., 2022; Razmjoue *et al*., 2023; Sulieman *et al*., 2023).

CONCLUSION

This study demonstrated that the essential oil of *M. officinalis* possesses potent antimicrobial properties against multi-drug resistant bacteria, particularly *P. aeruginosa* and *E. coli*. The MIC and MBC of *M. officinalis* essential oil were found to be 4 mg ml^{-1} and 8 mg ml^{-1}, respectively. This is significantly lower than the MIC and MBC of the majority of antibiotics tested, indicating that *M. officinalis* essential oil may be a more effective antimicrobial than the majority of antibiotics. Furthermore, the low MIC and MBC levels of *M. officinalis* essential oil suggest that it has specific antimicrobial properties even at low concentrations, making it a potential economical source of antimicrobials. This study has demonstrated the potential of *M. officinalis* essential oil as an alternative to conventional antibiotics, an attractive prospect given the growing prevalence of multi-drug resistant bacteria and the need for new antimicrobial solutions. Molecular docking studies have demonstrated that Caryophyllene oxide, a compound found in *M.officinalis* essential oil, has greater antimicrobial effects against *E. coli* bacteria when compared to other compounds. This was further corroborated by the results of disk diffusion, the MIC and MBC tests. As a result, *M. officinalis* essential oil is recommended for use as an oral antimicrobial agent in the food and medical industries due to its high efficacy against *E. coli* bacteria. Furthermore, its natural composition ensures that it is free from harmful chemicals, which could potentially cause adverse effects. Therefore, Caryophyllene oxide, found in *M. officinalis* essential oil, has the potential to be used as a safe and effective antimicrobial agent in the food and medical industries. Besides, the results of this research suggest that caryophyllene oxide is capable of binding to its receptor and inhibiting the enzyme, potentially making it a viable herbal compound for use with beta-lactamase antibiotics to reduce antibiotic resistance. However,

further research is needed to confirm these findings.

DECLARATIONS

Acknowledgment

The authors would like to express their gratitude to the R & D team of the Goldaru-co Pharmaceutical Laboratory in Iran and the Agricultural Research and Training Center, as well as the Natural Resources of Chaharmahal Va Bakhtiari province in Iran, for providing substantial support. Without their help, this study would not have been possible. The team's assistance enabled the authors to conduct a thorough investigation into the topic. This was a crucial factor in the successful completion of the research and its subsequent impact.

Funding

This study was supported by the authors, providing evidence of their commitment to furthering the research. Such an investment of time and effort is indicative of the authors' dedication to advancing knowledge in the field and demonstrates their confidence in the results of the study.

Ethics approval and consent to participate

The Ethical Council of the Islamic Azad University, Shahrekord Branch, approved the study of Research of the Medicinal Plants Processing Center. This research project was verified and the licenses related to the sampling process were approved by Dr. Mehrdad Ataie Kachoie and Prof. Fariborz Moattar (Approval Ref Number 2020-552). This approval demonstrates that the research study was conducted in accordance with the ethical principles of research and is compliant with international standards. Furthermore, this approval serves as a testament to the fact that the research team was granted permission to carry out the sampling process in a safe and responsible manner.

Data availability

All of manuscript datas are presented in this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abbasi, N., Akhavan, M.M., Rahbar-Roshandel, N. and Shafiei, M., 2014. The effects of low and high concentrations of luteolin on cultured human endothelial cells under normal and glucotoxic conditions: involvement of integrin-linked kinase and cyclooxygenase-2. *Phytother. Res.*, **28**: 1301-1307. https://doi.org/10.1002/ptr.5128

Abdellatif, F., Boudjella, H., Zitouni, A. and Hassani, A., 2014. Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian *Melissa officinalis* L. *Excli J.*, **13**: 772.

Abers, M., Schroeder, S., Goelz, L., Sulser, A., St Rose, T., Puchalski, K. and Langland, J., 2021. Antimicrobial activity of the volatile substances from essential oils. *BMC Complement. Med. Ther.*, **21**: 1–14. https://doi.org/10.1186/s12906-021-03285-3

Aidy, A., Karimi, E., Ghaneialvar, H., Mohammadpour, S. and Abbasi, N., 2020. Protective effect of Nectaroscordum tripedale extract and its bioactive component tetramethylpyrazine against acetaminophen-induced hepatotoxicity in rats. *Adv. Trad. Med.*, **20**: 471-477. https://doi.org/10.1007/s13596-020-00431-z

Ambreen, H., Aldaghfag, S.A., Yaseen, M., Iqbal, J., Zahid, M., Dahshan, A. and Hegazy, H.H., 2022. Electronic, optical and magnetic characteristics of V doped BeS. Phys. Scr., **97**: 065807. https://doi.org/10.1088/1402-4896/ac6910

Arzhang, M., Dakhili, M. and Farahani, F., 2015. Investigation of chemical compounds and anti-microbial activity of essential oil of *Melissa officinalis* L. *Qom Univ. Med. Sci. J.*, **9**: 7–13.

Bagherzadeh Lakani, F., Jalilpoor, J. and Masoumzadeh, M., 2024. Investigation of antioxidant capacity in farmed beluga (*Huso huso*) fry fed with *Echinacea purpurea* and garlic (*Allium sativum*) powder extracts. *Aqua Anim. Nutr.*, **5**: 1-9.

Basati, G., Khaksarian, M., Abbaszadeh, S., Lashgarian, H.E. and Marzban, A., 2019. Cancer stem cells and nanotechnological approaches for eradication. *Stem Cell Investig.*, **6**: 3. https://doi.org/10.21037/sci.2019.10.07

Darvishi, M., 2016. Antibiotic resistance pattern of uropathogenic methicillin-resistant staphylococcus aureus isolated from immunosuppressive patients with pyelonephritis. *J. Pure appl. Microbiol.*, **10**: 2663-2667. https://doi.org/10.22207/JPAM.10.4.24

Dorman, H. and Deans, S.G., 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J. appl. Microbiol.*, **88**: 308–316. https://doi.org/10.1046/j.1365-2672.2000.00969.x

Ebadollahi, A., Ashrafi, P.R. and Farjaminezhad, M., 2016. Phytochemistry, toxicity and feeding inhibitory activity of *Melissa officinalis* L. essential oil against a cosmopolitan insect pest; *Tribolium castaneum* Herbst. *Toxin Rev.*, **35**: 77–82. https://

doi.org/10.1080/15569543.2016.1199572

Ebrahimi, Y., Abdalkareem, J.S., Mohammed, B.A., Salman, A.N., Jabbar, A.M., Hameed, M.N., Goudarzi, M.A. and Parsaei, P., 2022. Determination of antioxidant properties of *Mentha longifolia*, *Pistacia khinjuk* and *Eucalyptus globulus*. *Caspian J. environ. Sci.*, pp. 1-6.

Ehsani, A., Alizadeh, O., Hashemi, M., Afshari, A. and Aminzare, M., 2017. Phytochemical, antioxidant and antibacterial properties of *Melissa officinalis* and *Dracocephalum moldavica* essential oils. In: *Veterinary Research Forum, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*, pp. 223.

Etame, R.E., Mouokeu, R.S., Pouaha, C.L.C., Igor, V.K., Tchientcheu, R., Assam, J.P., Monthe P.F.S. and Tiabou, A.T., 2018. Effect of fractioning on antibacterial activity of *Enantia chlorantha* Oliver (Annonaceae) methanol extract and mode of action. *Evid. Based Complement. Altern. Med. Ecam*, Article ID 4831593. https://doi.org/10.1155/2018/4831593

Ghamari, S., Abbaszadeh, S., Mardani, M. and Shahsavari, S., 2017. Identifying medicinal plants affecting the teeth from the Southern district of Ilam province, Iran. *J. pharmaceut. Sci. Res.*, **9**: 800.

Hamad, A., Djalil, A.D., Dewi, D.Y.S. and Hartanti, D., 2021. Development of lemon basil essential oil as a natural chicken meat preservative. In: *IOP conference series: Earth and environ. science*. IOP Publishing, pp. 12028. https://doi.org/10.1088/1755-1315/803/1/012028

Islam, N., Wang, H., Maqbool, F. and Ferro, V., 2019. In vitro enzymatic digestibility of glutaraldehyde-crosslinked chitosan nanoparticles in lysozyme solution and their applicability in pulmonary drug delivery. *Molecules*, **24**: 1271. https://doi.org/10.3390/molecules24071271

Jafari-Sales, A. and Pashazadeh, M., 2020. Study of chemical composition and antimicrobial properties of Rosemary (*Rosmarinus officinalis*) essential oil on *Staphylococcus aureus* and *Escherichia coli in vitro*. *Int. J. Life Sci. Biotechnol.*, **3**: 62–69. https://doi.org/10.38001/ijlsb.693371

Jafarzadeh, M.M., Moghaddam, M.J.M. and Bakhshi, D., 2020. Antimicrobial activity of three plant species against multi-drug resistant *E. coli* causing urinary tract infection. *J. Herb Med.*, **22**: 100352. https://doi.org/10.1016/j.hermed.2020.100352

Jalal, Z., El-Atki, Y., Lyoussi, B. and Abdellaoui, A., 2015. Phytochemistry of the essential oil of *Melissa officinalis* L. growing wild in Morocco: Preventive approach against nosocomial infections. *Asian Pac. J. trop. Biomed.*, **5**: 458–461. https://doi.org/10.1016/j.apjtb.2015.03.003

Jamal-Omidi, F., Mohajjel, S.H. and Sariri, R., 2018. Effect of water-deficit stress on secondary metabolites of *Melissa officinalis* L.: Role of exogenous salicylic acid. *Caspian J. environ. Sci.*, **16**: 121-134.

Khalili, G., Mazloomifar, A., Larijani, K. and Tehrani, M.S., 2018. Solvent-free microwave extraction of essential oils from *Thymus vulgaris* L. and *Melissa officinalis* L. *Ind. Crops Prod.*, **119**: 214–217. https://doi.org/10.1016/j.indcrop.2018.04.021

Khameneh, B., Iranshahy, M., Soheili, V. and Fazly-Bazzaz, B.S., 2019. Review on plant antimicrobials: A mechanistic viewpoint. *Antimicrob. Resist. Infect. Contr.*, **8**: 1–28. https://doi.org/10.1186/s13756-019-0559-6

Kiani, N. and Akbary, P., 2023. Effects of brown alga, Stoechospermum marginatum extract on growth performance, body chemical compositions and some serum biochemical parameters in grey mullet, *Mugil cephalus* (Linnaeus 1758). *Aqua Anim. Nutr.*, **9**: 15-29.

Klūga, A., Terentjeva, M. and Kántor, A., Kluz, M., Puchalski, C. and Kačániová, M., 2017. Antibacterial activity of *Melissa officinalis* L., *Mentha piperita* L., *Origanum vulgare* L. and *Malva mauritiana* against bacterial microflora isolated from fish. *Adv. Res. Life Sci.*, **1**: 75–80. https://doi.org/10.1515/arls-2017-0013

Lin, J.T., Chen, Y.C. and Lee, Y.C., 2012. Antioxidant, anti-proliferative and cyclooxygenase-2 inhibitory activities of ethanolic extracts from lemon balm (*Melissa officinalis* L.) leaves. *Fd. Sci. Technol.*, **49**: 1–7. https://doi.org/10.1016/j.lwt.2012.04.009

Marongiu, B., Porcedda, S., Piras, A., Rosa, A., Deiana, M. and Dessi, M.A., 2004. Antioxidant activity of supercritical extract of *Melissa officinalis* subsp. *officinalis* and *Melissa officinalis* subsp. inodora. *Phyther. Res.*, **18**: 789–792. https://doi.org/10.1002/ptr.1549

Mimica-Dukic, N., Bozin, B., Sokovic, M. and Simin, N., 2004. Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. *J. Agric. Fd. Chem.*, **52**: 2485–2489. https://doi.org/10.1021/jf030698a

Miraj, S., Rafieian-Kopaei and Kiani, S., 2017. *Melissa officinalis* L: A review study with an antioxidant prospective. *J. Evid. Based Complement. Altern. Med.*, **22**: 385–394. https://doi.org/10.1177/2156587216663433

Negahdari, B., Darvishi, M. and Saeedi, A.A., 2019.

Gold nanoparticles and hepatitis B virus. *Artif. Cells, Nanomed. Biotechnol*., **47**: 455-461. https://doi.org/10.1080/21691401.2018.1553786

Noshad, M., Hojjati, M. and Behbahani, B.A., 2018. Black Zira essential oil: Chemical compositions and antimicrobial activity against the growth of some pathogenic strain causing infection. *Microb. Pathog*., **116**: 153–157. https://doi.org/10.1016/j.micpath.2018.01.026

Pirbalouti, A.G., Nekoei, M., Rahimmalek, M. and Malekpoor, F., 2019. Chemical composition and yield of essential oil from lemon balm (*Melissa officinalis* L.) under foliar applications of jasmonic and salicylic acids. *Biocatal. Agric. Biotechnol*., **19**: 101144. https://doi.org/10.1016/j.bcab.2019.101144

Rădulescu, M., Jianu, C., Lukinich-Gruia, A.T., Mioc, M., Mioc, A., Şoica, C. and Stana, L.G., 2021. Chemical composition, in vitro and in silico antioxidant potential of *Melissa officinalis* subsp. *officinalis* essential oil. *Antioxidants*, **10**: 1081. https://doi.org/10.3390/antiox10071081

Razmjoue, D., Pirhadi, M., Soltanbeigi, A., Lysiuk, R. and Asadzadeh, R., 2023. Investigating the effect of total antioxidant capacity of medicinal plants *Salsola rigida* and *Triticum aestivum*. *Plant Biotechnol. Persa*, **5**: 1-6. https://doi.org/10.61186/pbp.5.2.1

Shahsavari, S., Sarkar, S., Sen, D.J. and Mandal, S.K., 2022. Determination of total antioxidant activity of methanolic extract of *Falcaria vulgaris*. *J. Biochem. Phytomed*., **1**: 8–12. https://doi.org/10.34172/jbp.2022.3

Shakeri, A., Sahebkar, A. and Javadi, B., 2016. *Melissa officinalis* L. A review of its traditional uses, phytochemistry and pharmacology. *J. Ethnopharmacol*., **188**: 204–228. https://doi.org/10.1016/j.jep.2016.05.010

Sulieman, A.M., Al-Anaizy, E.S., Al-Anaizy, N.A., Abdulhakeem, M.A. and Snoussi, M., 2023. Assessment of antimicrobial and antioxidant activity of methanolic extract from *Arnebia decumbens* aerial parts growing wild in Aja mountain. *Adv. Life Sci*., **10**: 84-92.

Szliszka, E., Czuba, Z.P. and Domino, M., 2009. Ethanolic extract of propolis (EEP) enhances the apoptosis- inducing potential of trail in cancer cells. *Molecules*, **14**: 738–754. https://doi.org/10.3390/molecules14020738

Tacconelli, E., 2017. *Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development*. World Health Organization. pp. 3.

Tsai, M.C., Huang, C.C., Wei, Y.C., Liu, T.T., Lin, M.T., Yi, L.N. and Tai, M.H, 2020. Combined chibby and β-catenin predicts clinical outcomes in patients with hepatocellular carcinoma. *Int. J. mol. Sci*., **21**: 2060. https://doi.org/10.3390/ijms21062060

Widoyo, H., Mohammed, Z.Y., Ramírez-Coronel, A.A., Iswanto, A.H., Thattarauthodiyil, U., Alkhayyat, A.S., Karimi, M., Bahmani, M. and Eftekhari, Z. 2023. Herbal therapy in Covid-19: A systematic review of medicinal plants effective against Covid-19. *Caspian J. environ. Sci.*, **21**: 1289-1298.

Yu, H., Pei, J., Qiu, W., Mei, J and Xie, J., 2022. The antimicrobial effect of *Melissa officinalis* L. essential oil on vibrio parahaemolyticus: Insights based on the cell membrane and external structure. *Front Microbiol*., **13**: 812792. https://doi.org/10.3389/fmicb.2022.812792