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Evaluation of chemical composition and antibacterial activity of methanolic extracts from nine medicinal plants against standard bacterial strains *in vitro*

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ABSTRACT: This research aims to identify the antibacterial properties of medicinal plants, which are crucial in traditional medicine due to their natural origin and high safety. Nine medicinal plants from East Azerbaijan Province were gathered for experimental-laboratory investigation, with essential oils extracted and gas chromatography-mass spectrometry analysis used to identify active components. Additionally, methanolic extracts were prepared at different concentrations using the Soxhlet method, and their effects on bacteria were evaluated through the agar well diffusion method, as well as MIC and MBC tests. At 400 mg mL^{-1}, all plant extracts in this investigation exhibited the strongest antibacterial activity. When it came to Gram-positive bacteria like *Staphylococcus aureus* and *Bacillus cereus*, the antibacterial activity of all plant extracts was noticeably greater than that of Gram-negative bacteria like *Pseudomonas aeruginosa* and *Escherichia coli*. The extracts of *Glycyrrhiza glabra*, *Mentha piperita* L., *Rosa damascena*, *and Peganum harmala* showed the strongest effects against Gram-positive bacteria, according to an evaluation of MIC and MBC values. The findings show that all plant methanolic extracts exhibit strong antibacterial properties against Gram-positive bacteria and may be viable therapeutic options. Furthermore, the essential oils of the plants under study included 209 different chemicals.
Keywords: medicinal plants; antibacterial activity; pathogenic bacteria; Gram-positive bacteria; Gram-negative bacteria.

Avaliação da composição química e atividade antibacteriana de extratos metanólicos de nove plantas medicinais contra cepas bacterianas padrão in vitro

RESUMO: Esta pesquisa visa identificar as propriedades antibacterianas de plantas medicinais, cruciais na medicina tradicional devido à sua origem natural e alta segurança. Nove plantas medicinais da Província do Azerbaijão Oriental foram coletadas para investigação experimental-laboratorial, com extração de óleos essenciais e análise por cromatografia gasosa e espectrometria de massas para identificar os componentes ativos. Além disso, extratos metanólicos foram preparados em diferentes concentrações pelo método de Soxhlet, e seus efeitos sobre as bactérias foram avaliados pelo método de difusão em ágar, bem como pelos testes de CIM e CBM. Na concentração de 400 mg mL^{-1}, todos os extratos vegetais desta investigação apresentaram a atividade antibacteriana mais forte. Em relação a bactérias Gram-positivas, como *Staphylococcus aureus* e *Bacillus cereus*, a atividade antibacteriana de todos os extratos vegetais foi notavelmente maior do que a de bactérias Gram-negativas, como *Pseudomonas aeruginosa* e *Escherichia coli*. Os extratos de *Glycyrrhiza glabra*, *Mentha piperita* L., *Rosa damascena* e *Peganum harmala* apresentaram os efeitos mais fortes contra bactérias Gram-positivas, de acordo com uma avaliação dos valores de CIM e CBM. Os resultados mostram que todos os extratos metanólicos de plantas apresentam fortes propriedades antibacterianas contra bactérias Gram-positivas e podem ser opções terapêuticas viáveis. Além disso, os óleos essenciais das plantas estudadas continham 209 substâncias químicas diferentes.
Palavras-chave: plantas medicinais; atividade antibacteriana; bactérias patogênicas; bactérias Gram-positivas; bactérias Gram-negativas.

1. INTRODUCTION

In many regions of the world, particularly in developing countries, the use of herbal compounds as a traditional therapeutic method for various diseases is widespread. This approach reflects the experiences and knowledge of past generations in utilizing natural resources to promote health

and treat diseases (JAFARI-SALES; HOSSEIN-NEZHAD, 2020; JAFARI-SALES; PASHAZADEH, 2020b).

Throughout all human civilizations, there has been a profound connection between humans and plants. However, many plant species with potential medicinal properties remain understudied and unidentified. Moreover, the discovery and utilization of new and valuable plant resources is a time-consuming process that requires extensive and meticulous research (JAFARı-SALES; PASHAZADEH, 2020a; YAMCHLOU *et al*., 2024).

Only a tiny portion of the abundant supply of advantageous chemical compounds found in plants has been used so far. Beyond their direct use as medicine, plants can provide unique templates for the design and development of pharmaceutical analogs. Additionally, the study of these plant compounds serves as a valuable tool for gaining deeper insights into biological processes and mechanisms related to health and disease (JAFARI-SALES; HOSSEIN-NEZHAD; et al., 2019; JAFARI-SALES; MOBAIYEN; et al., 2019; JAFARI-SALES; SHADI-DIZAJI, 2019; MOBAIYEN; JAFARI-SALES, 2019).

The overuse of chemical drugs has led to the emergence of antibiotic resistance in bacterial species (JAFARı-SALES; PASHAZADEH, 2020a). Medicinal plants, due to their natural origin, high safety, low cost, and affordability, are more popular than antibiotics. Worldwide, medicinal plants are used for therapeutic purposes to prevent, alleviate, or treat diseases. This approach has gained widespread attention not only due to its efficacy but also because of its accessibility and fewer side effects (JAFARI-SALES et al., 2017; SAYYAHI et al., 2021).

Plants produce compounds with specific antimicrobial properties, including alkaloids, flavonoids, isoflavonoids, tannins, glycosides, and phenolic compounds. The secondary metabolites in plants exhibit significant effects in combating microbes (MAHMOUDI et al., 2019). Infectious diseases are currently recognized as a serious global health concern, accounting for one-third of all deaths worldwide. Herbal antimicrobial drugs possess considerable therapeutic potential and reduce many associated side effects (SAGHAFI et al., 2021).

*Rosa damascena (*R. *damascena)*, belonging to the Rosaceae family, contains bioactive compounds such as anthocyanins, glycosides, and flavonoids(AMIRI et al., 2025). The extract of this plant has multiple therapeutic properties, including antiviral, antibacterial, anticancer, memory-enhancing, cardioprotective, and digestive-improving effects. Due to its diverse bioactive compounds, *R. damascena* extract is considered a promising natural resource for antimicrobial treatments (AGGARWAL *et al*., 2025; PEERAN *et al*., 2024; SAGHAFI *et al*., 2021).

Carum copticum (C. copticum) belongs to the Apiaceae family (BOSKABADY et al., 2014). It is effective in treating pharyngitis and possesses valuable therapeutic properties, including antioxidant, antiparasitic, expectorant, and anti-inflammatory effects. Numerous plant species benefit from the beneficial secondary metabolites that this plant generates, including lignans, flavonoids, and polyphenols (HEYDARZADEH et al., 2025).

Peganum harmala L. (*P. harmala* L.) is a member of the Zygophyllaceae family (ZHU et al., 2024). Extracts of this plant exhibit a range of biological properties, including antibacterial, antifungal, antiviral, cardioprotective, antitumor, and neuroprotective effects. Some of its compounds, such as β-carboline alkaloids (particularly harmine), have demonstrated profound anticancer properties (REJIEPU et al., 2025).

Salvia verticillata L. (*S. verticillata* L.) belongs to the Lamiaceae family (AĆIMOVIĆ *et al*.). Significant levels of polyphenolic chemicals, which have a wide range of biological effects such as antioxidant, anti-inflammatory, neuroprotective, anticancer, and metabolic advantages, are found in different species of this plant (LUCA et al., 2023).

Mentha piperita L. (*M. piperita* L.), a member of the Lamiaceae family (SINGH et al., 2015), contains bioactive compounds such as rutin, eugenol, apigenin, α-tocopherol, flavonoids, betaine, and ascorbic, oleanolic, caffeic, rosmarinic, and ursolic acids. These compounds exhibit anticancer, antiviral, and antihistaminic effects. Additionally, this plant has other therapeutic properties, including anti-inflammatory, analgesic, diuretic, blood pressure regulation, and urea reduction effects (PÉREZ-BERMÚDEZ et al., 2025).

Camellia sinensis (C. sinensis) belongs to the Theaceae family and contains diverse compounds, including polyphenols and antioxidants. It can prevent cardiovascular diseases by reducing oxidative stress, inflammation, platelet proliferation, and aggregation (JIBOLA-SHITTU et al., 2024).

Stachys schtschegleevii (*S. schtschegleevii*) is a member of the Lamiaceae family (HAZRATI et al., 2020). This plant has various medical properties, including antibacterial and antimicrobial effects, as well as applications in treating asthma, sinusitis, colds, rheumatism, and ear infections. It is also known as an anti-inflammatory and urinary tract disinfectant and is used for respiratory infections. Due to these properties, some refer to it as a natural "herbal penicillin" (NASROLLAHI et al., 2019).

Glycyrrhiza glabra (G. glabra) belongs to the Fabaceae family(EGHLIMA *et al*., 2025). Active ingredients in this plant, such as glycyrrhizin, glabridin, and isoliquiritigenin, are very useful in the treatment of several illnesses, such as hemophilia, chronic hepatitis B and C, acute liver problems, and immune system disorders (AFSHARZADEH et al., 2025).

Matricaria chamomilla (M. chamomilla) is a member of the Asteraceae family. The essential oil of different species of this plant can enhance the sensitivity of *Staphylococcus aureus* (*S. aureus*) to antibiotics by altering bacterial cell membrane permeability, thereby exhibiting anti-inflammatory and antibacterial properties. Biological properties of this plant include anti-inflammatory, anti-bacterial, antifungal, antioxidant, and anti-cancer properties (TAI et al., 2025). It has been used traditionally as a calming and sedative for a very long time (KAZEMI et al., 2024).

Investigating the antibacterial qualities of methanolic extracts of the aforementioned plants against standard strains in vitro is the aim of this investigation.

2. MATERIALS AND METHODS

2.1. Collection and extraction of vegetarian samples

This experimental-laboratory study was conducted at the research laboratory of the Islamic Azad University, Tabriz Medical Sciences Branch, on standard bacterial strains of *S. aureus* (American Type Culture Collection, ATCC: 25923), *Bacillus cereus* (*B. cereus*) (ATCC: 1247), *Pseudomonas aeruginosa (P. aeruginosa)* (ATCC: 27853), and *Escherichia coli (E. coli)*

(ATCC: 25922). The microorganisms used in this study were obtained as lyophilized cultures from the microbial collection of the University of Tehran, all characterized by ATCC specifications.

The aerial parts of the plants *R. damascena*, *C. copticum*, *P. harmala*, *S. verticillata L., M. piperita L.*, *C. sinensis*, *S. schtschegleevii*, *G. glabra*, and *M. chamomilla* were collected during spring from areas around cities in East Azerbaijan Province. Botanists from Islamic Azad University's Tabriz Branch recognized the plant specimens at the genus and species levels. The plant samples were pulverized into a powder after being allowed to dry in the shade. A Clevenger device was used to extract essential oils to identify the plants' active ingredients. After being dried with sodium sulfate, the resulting essential oil was passed through a 0.45 µm microfilter. Before chemical analysis, it was kept at 4°C in a dark glass container.

By comparing retention indices and mass spectra with standard mass spectra and trustworthy references, the components of the essential oils were determined. To accomplish full separation of the essential oil components, a suitable temperature program was chosen for the column. The mass spectra of the components were then acquired when the essential oil was put into a Gas Chromatography-Mass Spectrometry (GC/MS) apparatus (Agilent, USA). Using the Soxhlet technique, 300 g of each plant powder was extracted with 500 mL of methanol as the solvent at 40°C for 8 hours to create the methanolic extract (Merck, Germany). A rotary evaporator was then used to remove the methanol, and the extracts were kept in a refrigerator in dark containers until they were needed.

As the solvent for the well diffusion test, 5% dimethyl sulfoxide (DMSO) (Merck, Germany) was used to concentrate the extracts to concentrations of 50, 100, 200, and 400 mg mL^{-1}.

2.2. Determination of antibacterial effect of extract

One day before the experiment, the microorganisms under study were cultivated independently on Mueller-Hinton Agar (MHA) (Merck, Germany). Fresh bacterial colonies were moved to MHA in order to make the bacterial suspension. The microbial solution was diluted at a ratio of 0.01 to bring its turbidity down to the 0.5 McFarland standard, which is equal to 1.5 x 10^6 bacteria per mm. The agar well diffusion technique was used to assess the antibacterial efficacy of the methanolic extracts. 500 µL of the bacterial solution, or 1.5 x 10^6 bacteria per mm, was swabbed onto MHA plates in three different orientations using this technique. After that, wells of 6 mm in diameter and 5 mm in depth were punched at the proper intervals (2.5 cc apart). To the appropriate wells, 100 µL of each extract concentration was applied. Streptomycin (Padtan-Teb, Iran) was used as the positive control, while 5% DMSO was employed to create the negative control. The diameter of the inhibitory zones (in mm) was determined after a 24-hour incubation period at 37°C.

The tests were conducted three times for every extract and bacterial strain to guarantee dependability. Using serial dilutions of 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, and 200 mg mL^{-1}, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays were conducted in Mueller-Hinton Broth (MHB) (Merck, Germany) and MHA. The negative control was sterile media, whereas the positive control was bacterial colonies devoid of extract. Tubes exhibiting no turbidity were designated as the MIC after being incubated for 24 hours at 37°C. Samples were streaked onto MHA plates from the three tubes that came before the MIC. Plates that showed no signs of bacterial growth after 24 hours of incubation at 37°C were deemed the MBC. Every experiment was conducted three times to reduce experimental error.

2.3. Statistical analysis

SPSS software (version 26, SPSS Inc., Chicago, IL, USA) was used to analyze the data.

3. RESULTS

3.1. Essential oil compounds

Based on the chemical analysis of the essential oils of the studied plants by GC/MS, 15, 15, 28, 31, 21, 25, 28, 21, and 25 compounds were identified in the essential oils of *R. damascena*, *C. copticum*, *P. harmala*, *S. verticillata*, *M. piperita*, *C. sinensis*, *S. schtschegleevii*, *G. glabra*, and *M. chamomilla*, respectively, accounting for 99.8%, 98.1%, 94.4%, 95.8%, 97.1%, 82.45%, 87.7%, 88.7%, and 98.9% of the total essential oils.

The main compounds of the essential oils were identified as follows: In *R. damascena*, citronellol (26.7%) and geraniol (19%) were the dominant compounds. In *C. copticum*, thymol (36.9%), γ-terpinene (27.1%), and p-cymene (22.3%) constituted the major components. In *P. harmala*, eugenol (27.9%) and n-tetradecanol (12.3%) were identified as the principal compounds. In *S. verticillata*, germacrene D (14.2%) and spathulenol (11.3%) showed the highest abundance. In *M. piperita*, menthol (35.9%) and L-menthone (28.4%) were the predominant compounds. In *C. sinensis*, nonadecane (18.1%) and heneicosane (12.7%) had the highest share in the essential oil composition. In *S. schtschegleevii*, spathulenol (11.6%) and hexanoic acid (9.8%) were the main compounds. In *G. glabra*, isoniazid (15.1%) and methacrylonitrile (9.7%) were the most abundant. In *M. chamomilla*, (E)-β-farnesene (29.1%) and (E,E)-α-farnesene (10.3%) were the dominant compounds(Table 1). These compounds were recognized as the primary and active constituents of the essential oils, playing a significant role in the biological and therapeutic properties of these plants.

Table 1. Active compounds of plants using GC-MS.
Tabela 1. Compostos ativos de plantas usando GC-MS.

Rosa damascena		
No	Compounds	Percentage of compounds (%)
1	phenyl ethyl alcohol	10.2
2	citronellol	26.7
3	geraniol	19
4	geranial	1.2
5	citronellyl acetate	0.8
6	geranyl acetate	4.2
7	ß-caryophyllene	1.8
8	a-humulene	0.6
9	a- muurolene	0.4
10	n-heptadecane	2.1
11	)E,E)-farnesol	5.8
12	hexadecane	1.3
13	n-nonadecane	15.2
14	n-eicosane	2.4
15	n- henicosane	8.1
Total Identified Constituents		99.8

Carum copticum		
1	Sabinene	0.9
2	δ-3-carene	0.3
3	p-cymene	22.3
4	α-pinene	0.5
5	α-thujene	0.8
6	α -terpinene	1.3
7	Limoinene	2.3
8	γ-terpinene	27.1
9	β-pinene	1.4
10	Myrcene	0.7
11	terpinen-4-ol	0.1
12	methyl ether carvacrol	0.9
13	Thymol	36.9
14	Carvacrol	0.5
15	methyl ether thymol	2.1
	Total Identified Constituents	98.1

Peganum harmala L.		
1	epi-α-Bisabololo	3.4
2	Spathulenol	2.1
3	Methyl butanoate,3-methyl-3-butenyl	2.5
4	2E,4E-Dodecandienal	2.1
5	2-Acetyl-Thiazole	1.4
6	1-Esadecene	1.3
7	Decanoic acid	1.1
8	Camphor	2.4
9	Santolina alcohol	2.1
10	Dihydro carveol acetate	1.1
11	(Z)-Nerolidol	1.5
12	Dodecanoic acid	6.1
13	Eugenol	27.9
14	n-Undecanol	2.3
15	1-Octen-ol	1.1
16	Ciperotundone	3.4
17	Terpinyl acetate	0.8
18	Benzene acetonitrile	1.3
19	cis-Dihydro-rose oxide	2.1
20	α-Terpinen-7-al	2.1
21	(E)-Methyl isoeugenol	1.4
22	Methyl p-tert-buthylphenil acetate	3.9
23	3-Decanone	1.2
24	Cubenol	1.4
25	β-Curcumene	2.1
26	Longifolol	2.8
27	n-Octanol	1.2
28	n-Tetradecanol	12.3
	Total Identified Constituents	94.4

Salvia verticilata L.		
1	Spathulenol	11.3
2	Limonene	4.6
3	β -Cubebene	1.5
4	Cyercene	1.9
5	β-Pinene	2.9
6	1.8-Cineole	4.1
7	Sabinen	2.1
8	β -Caryophyllene	1.8
9	Naphthalane	3.3
10	δ -3-Carene	2.5
11	α -Eudesmol	1.8
12	Salvial-4 (14)-en-1-one	1.6
13	cis-Calamenene	1.4
14	2-Pentadecanone	1.8
15	β -Copaene	4.1
16	γ -Cadinene	1.5
17	Germacrene D	14.2
18	α-Pinene	3.1
19	Valeranone	3.2
20	Methyl ısoeugenol	1.5
21	(+)-Epi-bicyclosesquiphallendrene	1.3
22	Eudesma-4 [15], 7-dien-1-beta-ol	2.1
23	Caryophyllene oxide	2.1
24	p-Cymene	2.9
25	β-Bourbonene	2.3
26	Isolongifolene	1.8
27	Ethanone	2.7
28	α -Cubebene	2.1
29	δ -Cadinene	3.2
30	Bicyclogermacrene	3.2
31	α-Copaene	1.9
	Total Identified Constituents	95.8

Mentha piperita L.		
1	β - cubebene	2.3
2	carveone	1.8
3	Neomenthol	3.9
4	Trans caryophyllene	1.5
5	Limonene	2.6
6	β -pinene	1.6
7	Menthofuran	2.9
8	β - fenchyl alcohol	1.4
9	β-Bourbonene	0.3
10	Methyl acetate	3.3
11	Menthole	35.9
12	Trans-anethole	1.3
13	L -menthone	28.4
14	Caryophyllene oxide	2.5
15	Veridiflorol	2.7
16	Trans beta farnesene	0.6
17	1,8-cineole	2.1
18	Piperitone	1.1
19	bicyclogermacrene	0.5
20	n-decyl acetate	0.2
21	Dihydrocarveol acetate	0.2
	Total Identified Constituents	97.1

Camellia sinensis		
1	Caffeine	9.7
2	Benzoyl bromide	5.1
3	Linalool	1.1
4	1S-α-Pinene	2.1
5	Eicosane	0.4
6	2-Nonanone	0.3
7	Temazepam	2.1
8	Docosane	2.4
9	Heptadecane,8-methyl	1.1
10	2-(4-Fluorophenyl)-1H-indole-5-carbaldehyde	1.2
11	9-Thiabicyclo [3.3.1]non-7-en-2- ol	2.8
12	Tricosane	4.8
13	Bis(2-ethylhexyl) phthalate	0.4
14	Acetophenone	1.1
15	Heneicosane	12.7
16	Nonanal	0.8
17	2-(1-Hydroxynaphthyl-2)quinolin	2.4
18	Dibutyl phthalate	5.3
19	Octadecanal	1.2
20	2-Nonadecanone	1.3
21	Tetracosane	2.45
22	Phytol	1.6
23	Cedryl propyl ether	0.9
24	Octadecane	1.1
25	Nonadecane	18.1
	Total Identified Constituents	82.45

	Stachys schtschegleevii	
1	β-Pinene	0.9
2	Valencene	8.1
3	1,18-Octadecadienal	1.9
4	Hexanoic acid	9.8
5	Salvial	1.3
6	Bis(2-furyl)methylvinylsilane	1.7
7	α-Cadinol	1.7
8	α-Amorphene	0.5
9	α-Pinene	1.1
10	2,6-Octadiene-1-ol,3,7-dimethyl	3.1
11	9,12-Octadecadienoic acid	2.9
12	α-Copaene	0.6
13	Globulol	2.1
14	Hexadecane-2,6,10,14-tetramethyl	3.1
15	1 ,18-Octadecandioic acid	1.9
16	Tau-Muurolol	2.7
17	1,2-Benzendicarboxylic acid	0.7
18	3,4-Difluoro-4-methoxybiphenyl	2.3
19	δ-Cadinene	4.2
20	β-Elemene	1.3
21	Spathulenol	11.6
22	Minsulfide	2.2
23	α-Cadinol	4.1
24	Phenol,2,5,5-trimethyl	0.9
25	γ-Eudesmol	0.2
26	2-Pentadecanone,6,10,14-trimethyl	8.4
27	Spathulenol	3.2
28	Germacrene- D	5.2
	Total Identified Constituents	87.7

	Glycyrrhiza glabra	
1	Benzene	4.5
2	Linalool	2.8
3	Diethyltoluamide	7.9
4	Methacrylonitrile	9.7
5	Phenol, 2-methoxy-4-(1-propenyl)-, (E)	1.4
6	trans-Permethrin	1.1
7	Benzoic acid	5.5
8	Isoniazid	15.1
9	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	2.4
10	2-Propenenitrile, 2-methyl	7.1
11	Pyrazine, 1,4-dioxide	4.4
12	Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl	2.1
13	4-Pyridinamine	1.9
14	Phenol, 4-(2-aminopropyl)	2.4
15	Retinol	1.8
16	Ethylenimine	1.7
17	Hydrochlorothiazide	4.9
18	Iodoquinol	1.9
19	Prasterone	5.2
20	Aspartic acid	2.3
21	Warfarin	2.6
	Total Identified Constituents	88.7

	Matricaria chamomilla	
1	Chamazulene	6.6
2	α-Bisabolol oxide B	6.1
3	(E)-β-Farnesene	29.1
4	Artemisia ketone	0.7
5	(E)-Spiroether	1.1
6	Menthol	0.7
7	Germacrene D	6.4
8	δ-Elemene	1.1
9	α-Bisabolol	2.2
10	(Z)-Spiroether	5.5
11	γ-Terpinene	0.8
12	(E)-Nerolidol	5.5
13	1,8-Cineole	0.3
14	(E)-Caryophyllene	0.8
15	Limonene	0.9
16	Bicyclogermacrene	0.8
17	Bicyclogermacrene	1.7
18	Myrcene	0.8
19	α-Bisabolol oxide A	8.1
20	α-Thujene	0.3
21	(E)-β-Ocimene	3.1
22	Sabinene	0.8
23	(E,E)-α-Farnesene	10.3
24	α-Pinene	1.9
25	α-Bisabolone oxide A	3.3
	Total Identified Constituents	98.9

3.1. The antibacterial properties of methanolic extracts from nine plants

In this study, the antibacterial properties of methanolic extracts from nine plants, *R. damascena*, *C. copticum*, *P. harmala*, *S. verticillata* L., *M. piperita* L., *C. sinensis*, *S. schtschegleevii*, *G. glabra*, and *M. chamomilla,* were evaluated against standard strains of Gram-positive (*S. aureus* and *B. cereus*) and Gram-negative (*P. aeruginosa* and *E. coli*) bacteria under laboratory conditions. The examined extracts showed strong antibacterial activity, according to the outcomes of the well diffusion, MIC, and MBC tests.

All plant extracts showed significant antibacterial activity against both Gram-positive and Gram-negative bacteria, according to the well diffusion experiment. However, compared to Gram-negative bacteria (*P. aeruginosa* and *E. coli*), their action against Gram-positive bacteria (*S. aureus* and *B. cereus*) was noticeably stronger. This discrepancy is most likely caused by the structural variations in bacterial cell walls: Gram-negative bacteria have an extra outer membrane with lipopolysaccharides that acts as a protective barrier and makes penetration more challenging, whereas Gram-positive bacteria have a thick peptidoglycan layer that facilitates the penetration of antibacterial compounds.

As the extract concentration increased, the bacterial growth inhibition also significantly increased, with maximum effects observed at 400 mg mL^{-1}. This concentration was considered the peak of antibacterial activity, where the extracts could completely inhibit bacterial growth. Among the tested extracts, *G. glabra* and *P. harmala* showed the largest inhibition zones (27.33 mm) at 400 mg mL^{-1}against *S. aureus*, indicating their stronger bioactive compounds, while in contrast, *R. damascena*, *C. copticum*, *S. verticillata*, *C. sinensis*, and *M. chamomilla* exhibited the smallest inhibition zones at lower concentrations (50-100 mg mL^{-1}), with no inhibition zones observed for *E. coli* and *P. aeruginosa* at 50 mg mL^{-1} (Table 2).

The MIC and MBC results indicated that *G. glabra*, *M. piperita*, *R. damascena*, and *P. harmala* had the strongest effects on Gram-positive bacteria but minimal impact on Gram-negative bacteria, with *G. glabra* and *M. piperita* exhibiting the highest antibacterial activity against Gram-positive bacteria. At the same time, *R. damascena* and *P. harmala* also showed significant activity, though less effective against Gram-negative strains (Table 3).

4. DISCUSSION

Bacterial resistance to antibiotics is one of the most critical global health challenges, making the identification of alternative treatment methods essential. Medicinal plants, due to their diverse therapeutic properties, including antibacterial

effects, have always been a focus of researchers. In recent years, extensive studies have been conducted on the characteristics of these natural compounds, demonstrating that plant essential oils and extracts can serve as suitable alternatives to antibiotics (KÜREKCI; BEYAZIT, 2022; PATIL et al., 2023). The present study has shown that methanolic extracts from the nine examined plants exhibit significant antibacterial effects against pathogenic bacteria such as *S. aureus*, *B. cereus*, *E. coli*, and *P. aeruginosa* under laboratory conditions.

Table 2. Diameter of bacterial inhibition zones (mm) at different concentrations of the studied plant methanolic extracts.
Tabela 2. Diâmetro das zonas de inibição bacteriana (mm) em diferentes concentrações dos extratos metanólicos das plantas estudadas.

Plants	Bacterial strain	Extract concentration (mg mL^{-1})					
		50	100	200	400	Negative control	Positive control
Rosa damascena	*S. aureus*	13.33±0.57	18±1.73	20.33±0.57	26.66±0.57	-	23
	B. cereus	9.33±0.57	14.66±1.52	18.33±0.57	23±1.73	-	20
	E. coli	0	10.33±0.57	16±1.73	19.33±0.57	-	25
	P. aeruginosa	0	0	8.66±1.52	10.66±1.15	-	22
Carum copticum	*S. aureus*	9.33±0.57	15.33±0.57	18.33±0.57	20.66±1.15	-	23
	B. cereus	0	7.66±1.52	11.66±2.08	17.22±1.52	-	20
	E. coli	0	0	7.66±0.57	12.33±1.15	-	25
	P. aeruginosa	0	0	0	6.66±1.15	-	22
Peganum harmala L.	*S. aureus*	9.66±1.52	15.66±0.57	20.66±1.52	27.33±1.15	-	23
	B. cereus	8.66±1.73	14.66±1.15	18.33±1.52	21.66±0.57	-	20
	E. coli	6.66±1.52	10.66±1.52	12.66±2.30	16.66±2.88	-	25
	P. aeruginosa	0	0	8.66±1.15	11.66±1.15	-	22
Salvia verticillata L.	*S. aureus*	9.33±1.52	12.33±1.52	16±1	19.33±1.52	-	23
	B. cereus	8.66±0.57	11.66±2.08	15.33±0.57	16.66±1.15	-	20
	E. coli	0	9.33±0.57	12.66±2.08	11.66±1.52	-	25
	P. aeruginosa	0	0	9.33±0.57	10.66±0.57	-	22
Mentha piperita L.	*S. aureus*	19.33±0.57	21.33±1.15	23.33±0.57	25.66±1.15	-	23
	B. cereus	18.33±0.57	20.66±1.15	22.33±0.57	24.33±0.57	-	20
	E. coli	15.33±1.52	16.33±1.52	17.66±1.15	18.66±2.08	-	25
	P. aeruginosa	9.33±0.57	10.66±1.15	11.66±1.15	12.66±0.57	-	22
Camellia sinensis	*S. aureus*	8.33±0.57	12.66±0.57	17.66±1.52	21.33±1.15	-	23
	B. cereus	7.66±0.57	11.33±0.57	15.33±1.52	19.33±0.57	-	20
	E. coli	0	0	10.66±0.57	14.33±0.57	-	25
	P. aeruginosa	0	0	0	8.66±1.15	-	22
Stachys schtschegleevii	*S. aureus*	11.33±1.15	13.66±0.57	16.66±1.52	18.66±0.75	-	23
	B. cereus	9.33±0.57	11±1	13.33±0.57	16.66±1.52	-	20
	E. coli	7.66±0.57	10.66±1.52	12.66±1.15	14.66±1.15	-	25
	P. aeruginosa	0	6.33±0.57	8.66±1.15	10.66±1.15	-	22
Glycyrrhiza glabra	*S. aureus*	19.33±1.52	21.66±1.52	24.66±0.57	27.33±1.15	-	23
	B. cereus	15.33±1.15	16.66±1.52	18.33±0.57	19.33±0.57	-	20
	E. coli	13.33±0.57	14.33±0.57	15.33±0.57	16.33±1.52	-	25
	P. aeruginosa	7.66±1.15	8.33±0.57	10±1.73	11.33±0.57	-	22
Matricaria chamomilla	*S. aureus*	9.66±1.52	11.66±1.52	14.66±1.52	18.66±0.57	-	23
	B. cereus	8.66±1.52	10.33±2.08	13.66±1.15	19.33±1.52	-	20
	E. coli	0	7.33±0.57	10.66±1.52	13.33±0.57	-	25
	P. aeruginosa	0	0	8.33±0.57	10±1.73	-	22

In the study by Safdar; Malik (2020), *R. damascena* extract demonstrated strong antibacterial activity against *S. aureus* (Gram-positive) and *Enterobacter* (Gram-negative), attributed to the presence of flavonoids and terpenoids that damage bacterial cell walls and inhibit their growth. Niazi et al. (2025) found that the ethanolic extract of *R. damascena* exhibited notable activity against Gram-positive bacteria (*S. aureus* and *Bacillus subtilis* (*B. subtilis*)) and Gram-negative *E. coli*, with effects comparable to standard antibiotics in some cases, likely due to flavonoids and phenols. Ghavam (2024) research revealed that *R. damascena* essential oil possesses antibacterial properties, with 19 chemical compounds (98.96% of the total oil) identified through distillation, including oleic acid, palmitic acid, stearic acid, citronellol, and nonadecane.

According to Jafari-Sales et al. (2019), the methanolic extract of *C. copticum* possesses antibacterial properties against Gram-positive bacteria (*S. aureus* and *B. cereus*), suggesting that it might be used as an adjuvant treatment. Fallah et al. (2013) reported that *C. copticum* extracts significantly inhibited multidrug-resistant *Pseudomonas* (*S. aureus*), with efficacy observed even at low concentrations (6.25 mg mL^{-1}), suggesting their effectiveness against complex resistance mechanisms. Raja et al. (2016) identified *C. copticum* essential oil as a promising option against *S. aureus*, as its active compounds are both potent and safe.

Mohsenipour; Hassanshahian (2016) found that *P. harmala* extracts, particularly the ethanolic extract, had the strongest inhibitory effect on *S. aureus* and *B. cereus*. Bin-Masalam et al. (2021) tested *P. harmala* extract against five human pathogenic bacteria (*E. coli*, *Klebsiella pneumoniae* (*K. pneumoniae*), *P. aeruginosa*, *B. cereus*, and *S. aureus*), with the alcoholic extract showing the highest efficacy against *S. aureus*. According to Iranshahy et al. (2019), *P. harmala* extracts and chemical compounds have strong antibacterial properties, especially against Gram-positive bacteria like *Micrococcus luteus* (*M. luteus*) and *S. aureus*.

Table 3. MIC and MBC of methanolic extracts of plants on the tested bacteria in terms of (mg mL^{-1}).
Tabela 3. CIM e CBM de extratos metanólicos de plantas sobre as bactérias testadas em termos de (mg mL^{-1}).

Plants	Bacterial strain	MIC	MBC
Rosa damascena	*S. aureus*	6.25	12.5
	B. cereus	12.5	25
	E. coli	50	100
	P. aeruginosa	100	200
Carum copticum	*S. aureus*	12.5	25
	B. cereus	25	100
	E. coli	50	100
	P. aeruginosa	100	200
Peganum harmala L.	*S. aureus*	6.25	12.5
	B. cereus	12.5	25
	E. coli	50	100
	P. aeruginosa	100	200
Salvia verticillata L.	*S. aureus*	12.5	25
	B. cereus	25	50
	E. coli	50	100
	P. aeruginosa	100	200
Mentha piperita L.	*S. aureus*	6.25	12.5
	B. cereus	12.5	25
	E. coli	50	100
	P. aeruginosa	100	200
Camellia sinensis	*S. aureus*	12.5	25
	B. cereus	25	50
	E. coli	50	100
	P. aeruginosa	100	200
Stachys schtschegleevii	*S. aureus*	25	50
	B. cereus	50	100
	E. coli	100	200
	P. aeruginosa	100	200
Glycyrrhiza glabra	*S. aureus*	6.25	12.5
	B. cereus	12.5	25
	E. coli	50	100
	P. aeruginosa	100	200
Matricaria chamomilla	*S. aureus*	25	50
	B. cereus	50	100
	E. coli	100	200
	P. aeruginosa	100	200

Sreckovic et al. (2018) reported that the methanolic extract of *S. verticillata* L., rich in phenolic compounds, showed the strongest antimicrobial activity against *B. cereus*, with an MIC of 1.25 mg mL^{-1}(SREĆKOVIĆ *et al.*, 2018). Erbil; Digrak (2015) observed that *S. verticillata* L. methanolic extracts were effective against *B. subtilis* and *Enterobacter aerogenes* (*E. aerogenes)* but showed no activity against *E. coli*. Nasermoadeli et al. (2013) compared the essential oil composition of wild and cultivated *S. verticillata* L., identifying (E)-caryophyllene (17.8%), beta-phellandrene (14.2%), and alpha-humulene (10.2%) as major compounds in cultivated samples. In comparison, wild samples contained significant amounts of alpha-gurjunene (12.8%) and germacrene D (8.7%).

M. piperita L. methanolic extract, particularly at high concentrations, had a stronger inhibitory effect on Gram-positive bacteria (*S. aureus* and *B. cereus*) than on Gram-negative bacteria (*E. coli* and *P. aeruginosa*) (MAHMOUDI et al., 2019). In comparison to conventional antibiotics, Singh et al. (2015) found that *M. piperita* L. showed strong antibacterial activity against Gram-positive *S. aureus* and *Streptococcus pyogenes*, but reduced efficacy against Gram-negative *E. coli* and *K. pneumoniae*. Yazdani (2019) identified 17 compounds in *M. piperita* L. essential oil, with menthol, neomenthyl acetate, and menthofuran being the most abundant, showing stronger activity against Gram-positive bacteria.

With little effect on Gram-negative bacteria (*E. coli*, *Salmonella Typhi*, and *P. aeruginosa*), Chan et al. (2011) showed that *C. sinensis* extract had the strongest antibacterial action among the various tea varieties, especially against Gram-positive bacteria (*S. aureus*, *M. luteus*, and *B. cereus*) (CHAN *et al.*, 2011). Mehta et al. (2016) found that aqueous *C. sinensis* extract showed stronger antibacterial activity against *S. aureus* compared to *P. aeruginosa* and *E. coli*, unlike methanolic, ethanolic, and acetone extracts. Hope et al. (2022) identified flavonoids, terpenes, and alkaloids (including caffeine, 82.69%) as the main bioactive compounds responsible for its antibacterial, antioxidant, and anti-inflammatory properties.

Noshad (2020) observed that *S. schtschegleevii* ethanolic extract had stronger antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*, *P. aeruginosa*) bacteria than its aqueous extract, though Gram-positive bacteria were more sensitive(NOSHAD, 2020). Hamedfar et al. (2025) reported that the methanolic extract of *S. schtschegleevii* had a stronger inhibitory effect on *S. aureus* and *B. cereus* than ethanolic and aqueous extracts. Nasrollahi et al. (2019) confirmed its antibacterial, antioxidant, and anticancer properties, identifying spathulenol (11.67%) and germacrene D (25.68%) as major compounds(NASROLLAHI *et al.*, 2019).

Hojjati Bonab; Nikkhah (2012) found that *G. glabra* methanolic extract had significant antibacterial effects against enterobacteria (*E. coli*, *E. aerogenes*, and *K. pneumoniae*), though bacterial sensitivity varied minimally. Karahan et al. (2016) noted that *G. glabra* root extract inhibited Gram-positive *S. aureus* more effectively than Gram-negative *E. coli*, *P. aeruginosa*, and *K. pneumoniae*(KARAHAN *et al.*, 2016). Chouitah et al. (2011) identified 21 major compounds (70% of total content), including isoniazid (13.36%) and methacrylonitrile (9.69%).

Boudıeb et al. (2018) reported that *M. chamomilla* methanolic, aqueous, and particularly chloroform extracts inhibited *S. aureus* and *E. coli*. Methanolic and ethyl acetate extracts of *M. chamomilla* had exceptional antibacterial activity against *S. aureus* and *E. coli*, according to Mariod et al. (MARIOD *et al.*, 2019). According to Hameed et al. (2018), (E)-β-farnesene is a significant bioactive molecule that has great potential to combat *E. coli* and *P. aeruginosa*.

The discrepancy between the results of plant compounds and antibacterial properties with the findings of other researchers can be attributed to several factors, including differences in plant compound extraction methods, genetic

and geographical diversity of plants, variations in chemical analysis techniques, antibacterial testing conditions, interactions between plant compounds and synergism/antagonism, methodological and statistical errors (such as low number of replicates, lack of precise control over temperature and humidity during incubation, or errors in interpreting inhibition zones), and selective publication bias.

5. CONCLUSIONS

The chemical makeup of essential oils from nine distinct plant species, which are known to be the primary active ingredients in plant essential oils and are crucial to the biological and medicinal qualities of these plants, was assessed in this research. Furthermore, methanolic extracts from these plants were evaluated for their antibacterial properties against both Gram-positive and Gram-negative bacteria.

The findings indicate that the studied extracts exhibit significant antibacterial activity, with the most pronounced effects observed against Gram-positive bacteria. The use of these plant extracts as a natural source for creating new alternative antibacterial agents may be made possible by additional complementary research on animal models and clinical trials, given their high potential for bacterial growth inhibition and their lower side effect rates when compared to chemical drugs.

To accurately identify the active ingredients, improve extraction techniques, and assess their toxicity and adverse effects in human trials and animal models, further investigation is required.

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Data availability: Study data can be obtained by email from the corresponding author. It is not available on the website as the research project is still under development.

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