



Chemical Composition and Antimicrobial Activities of the Essential Oil From *Salvia mirzayanii* Leaves

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Abstract

Resistance of many pathogens to available drugs is a global challenge and is leading to growing interest in natural alternative products. In this study, chemical composition and in vitro antibacterial and antifungal activities of the essential oil from *Salvia mirzayanii* were investigated. The chemical constituents of essential oil from *S mirzayanii* were analyzed by gas chromatography–mass spectrometry. The antimicrobial activity was determined by broth microdilution. The main identified compounds were 1,8-cineole (41.2 ± 1.3%), linalool acetate (11.0 ± 0.5%), and α-terpinyl acetate (6.0 ± 0.4%) (mL of essential oil/g of plant material). The MIC₉₅ were 0.03 to 0.5 μL/mL and 16 to 128 μL/mL for gram-positive and gram-negative bacteria, respectively. These results indicated that *Salvia mirzayanii* essential oil significantly inhibited the growth of standard and clinically isolated tested yeasts by MIC₅₀ 0.03 to 1 μL/mL. Potent antibacterial and antifungal activities of *Salvia mirzayanii* essential oil may be considered in future study, particularly against antibiotic-resistant cases.

Keywords

Salvia mirzayanii, essential oil, antibacterial, antifungal, gas chromatography–mass spectrometry

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The remarkable rise of resistant microorganisms is a global challenge, and because of the increase of life-threatening cases in the clinic subsequently there is need nowadays to find novel and potent antimicrobial agents.^{1,2}

Among different bacteria, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* species, third-generation cephalosporin-resistant *Escherichia coli*, and resistant *Pseudomonas aruginosa* are the most resistant bacteria.³ Furthermore, *Candida albicans* was found to be the cause of two thirds of invasive candidiasis cases. *Candida dubliniensis* and *Candida glabrata* are more noticeable because of upward resistance to antifungal drugs.⁴

Folk medicinal plants could be proper sources for finding new antimicrobial compounds.⁵ It seems that natural antimicrobial components have different mechanisms in comparison to current antimicrobials and may be effective against resistant microbial strains in clinical cases.⁶ Plant essential oils are secondary metabolites present in different parts of plant. They possess several volatile components.^{1,7,8} According to previous studies, essential oils inhibit the growth of bacteria,

yeasts, and moulds¹; thus they are considered as natural antimicrobial agents.⁷

Salvia mirzayanii (*Moor-e-Talkh* in Persian) belongs to the Lamiaceae family and is endemic in the south of Iran.⁹ In folk medicine, the aerial parts of *Salvia mirzayanii* have been

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prescribed in the treatment of several disease such as diarrhea, stomachache, headache, diabetes, and hyperchlostermia in south of Iran.¹⁰

The purpose of this study was to determine the chemical components and in vitro antifungal and antibacterial activities of essential oil of *Salvia mirzayanii*.

Materials and Methods

Essential Oil Preparation

Aerial parts of *Salvia mirzayanii* were harvested before the flowering stage in September 2012 from southern regions of Iran, Bandar Abbas (Hormozgan province), and was identified and confirmed (Voucher No. 663) by Dr Mahmoodreza Moein in the Museum of Medicinal Plants, Department of Pharmacognosy, Shiraz University of Medical Sciences, Shiraz, Iran.

In brief, dried leaves of *Salvia mirzayanii* were ground in a grinder and 30 g of powder was hydrodistilled during 4 hours by Clevenger-type apparatus (yield 2.45%).

The obtained essential oils were dried over anhydrous sodium sulfate, filtered, stored at low temperature (4°C) until tested, and analyzed.

Essential oil analysis was performed using gas chromatography equipped with a mass spectrometer detector (Agilent Technologies Model 5975 C). The gas chromatograph was also provided a capillary column 60 m × 0.25 mm id, film thickness 0.25 mm. The oven program was as follows: temperature increase from 60°C at a rate of 5°C/min up to 250°C and finally held for 10 minutes. The transfer line temperature was 250°C. Helium was used as the carrier gas at a flow rate of 1.1 mL/min with a split ratio equal to 1/50. The quadrupole mass spectrometer was scanned over 35 to 465 amu with an ionizing voltage of 70 eV and an ionization current of 150 mA.

The injector and mass spectrometry transfer line temperatures were set at 250°C. Kovat's indices (KI) was determined by using retention times of *n*-alkanes (C9-C5) that were injected after the essential oil under the same chromatographic conditions. Tentative identification of the compounds was based on the comparison of their relative retention time and mass spectra with Wiley 275 and Adams data libraries for gas chromatography-mass spectrometry as well as those reported previously.^{10,11}

Determination of Antimicrobial Activities

Microorganisms. The antifungal activities of the essential oils against 15 standard strains of fungi, including *Candida albicans* (ATCC 5982, 1912, 562, 1905, 1949, 10261), *Candida tropicalis* (ATCC 750), *Candida krusei* (ATCC 6258), *Candida glabrata* (ATCC 863, 2192, 2175, 6144), *Candida dubliniensis* (CBS 8501, ATCC 8500), and *Candida parapsilosis* (ATCC 4344), were determined. In addition, the antifungal activities of the essential oil against 24 clinical isolates of yeasts identified by polymerase chain reaction-restriction fragment length polymorphism were also examined. The antifungal susceptibility of clinical isolates of the tested fungi were examined by microdilution and disk diffusion methods, and fluconazole was used as positive control in the same experimental conditions. The antibacterial activities of the essential oil against standard species of *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC11700), *Escherichia coli*, enterohemorrhagic *Escherichia coli* (ATCC 43894), *Streptococcus mutans* (ATCC 35668), *Streptococcus pneumonia* (ATCC 33400), *Streptococcus pyogenes* (ATCC 8668), *Salmonella enterica* (ATCC 14028), *Shigella flexneri* (NCTC 8516), and clinical isolates

Staphylococcus aureus, *Enterococcus faecium*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* collected from the Dr Faghihi Hospital (Shiraz, Iran) were also determined in this study.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentrations (MICs) were determined using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute with some modifications.^{12,13} Briefly, for determination of antifungal activities, serial dilutions of the essential oil (0.031-128 µL/mL) were prepared in 96-well microtiter plates using RPMI-1640 media (Sigma, St Louis, MO) buffered with MOPS (Sigma). To determine the antibacterial activities, serial dilutions of the compounds (0.031-128 µL/mL) were prepared in Muller-Hinton media (Merck, Darmstadt, Germany). For yeasts and bacteria, stock inoculums were prepared by suspending 3 colonies of the examined microorganisms in 5 mL sterile 0.85% NaCl, and adjusting the turbidity of the inoculums to 0.5 McFarland standards at 630 nm wavelength (this yields stock suspension of $1-5 \times 10^6$ CFU/mL for yeasts and $1-1.5 \times 10^8$ CFU/mL for bacteria). Working suspension was prepared by making a 1/1000 dilution of the stock suspension with RPMI or Muller-Hintonbroth for yeasts and bacteria, respectively. To each well of the microtiter plates, 0.1 mL of the working inoculums was added and the plates were incubated in a humid atmosphere at 30°C for 24 to 48 hours (fungi) or at 37°C for 24 hours (bacteria). Two hundred microliters of un-inoculated medium was included as a sterility control (blank). In addition, growth controls (medium with inoculums but without the essential oil) were also included. The growth in each well was compared with that of the growth control well. MICs were visually determined and defined as the lowest concentration of the compounds produced $\geq 50\%$ and $\geq 95\%$ growth inhibition for fungi and $\geq 95\%$ growth reduction for bacteria compared with the growth control wells. Each experiment was performed in triplicate.

In addition, media from wells with fungi showing no visible growth were further cultured on Sabouraud dextrose agar (Merck, Darmstadt, Germany) and from wells with bacteria showing no visible growth on Muller-Hinton agar (Merck, Darmstadt, Germany) to determine the minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC), respectively. MFCs and MBCs were determined as the lowest concentration yielding no more than 4 colonies, which corresponds to a mortality of 98% of the microorganisms in the initial inoculums.

Results

As indicated in Table 1, 13 compounds were identified in the oil. The main constituents of the oil were 1,8-cineole ($41.2 \pm 1.3\%$), linalool acetate ($11.0 \pm 0.5\%$), and α -terpinyl acetate ($6.0 \pm 0.4\%$). The percentage of monoterpene hydrocarbons (72.3%) was higher than sesquiterpene hydrocarbons (7.3%).

The antibacterial activities of *Salvia mirzayanii* essential oil are shown in Table 2. Essential oils inhibited the growth of all gram-positive bacteria at the concentrations of 0.03 to 0.5 µL/mL. The essential oils exhibited antibactericidal activity (MBC) against all the above-mentioned gram-positive bacteria at concentrations ranging from 0.062 to 32 µL/mL. The MBC of *Streptococcus pyogenes* ATCC 8668 was only in concentrations >0.031 µL/mL.

The growth of all gram-negative bacteria was inhibited by *Salvia mirzayani* essential oil at the concentration range of

Table 1. Chemical Composition of the Essential Oil From *Salvia mirzayanii*.

Peak No.	Components	KI (HP-5)	% in oil	Identification
1	Myrcene	991	4.7	MS, KI
2	1,8-Cineole	1033	41.2	MS, KI
3	cis-Ocimene	1039	1.5	MS, KI
4	Linalool	1130	2.5	MS, KI
5	Linalool acetate	1262	10.7	MS, KI
6	α -Terpinyl acetate	1353	5.7	MS, KI
7	Neryl acetate	1369	2.3	MS, KI
8	Geranyl acetate	1387	3.7	MS, KI
9	a-Gurjunene	1406	1.7	MS, KI
10	Aromadendrene	1439	1.7	MS, KI
11	α -Muurolene	1498	1.6	MS, KI
12	γ -Cadinene	1510	3.3	MS, KI
13	α -Cadinol	1658	0.72	MS, KI
	Identification		79.62	
	Sesquiterpene hydrocarbons		7.32	
	Alcoholic compounds		3.22	

Abbreviations: KI, Kovat's index; MS, mass spectrometry.

64 to 128 $\mu\text{L}/\text{mL}$ (MIC), except for *Shigella flexneri* NCTC 8516 and *Escherichia coli* ATCC 25922 (MIC 16 $\mu\text{L}/\text{mL}$). For gram-negative bacteria, the MBCs were the same for each given bacteria ($\geq 128 \mu\text{L}/\text{mL}$) except for third-generation cephalosporin-sensitive *Escherichia coli* (64 $\geq 128 \mu\text{L}/\text{mL}$) and *Shigella flexneri* NCTC 8516 (32 $\mu\text{L}/\text{mL}$).

The antifungal activities of *Salvia mirzayanii* essential oil against fungi are exhibited in Table 3. For the clinical and standard tested yeasts MIC₅₀ and MIC₉₅ for the *Salvia mirzayani* essential oil were in the range of 0.03 to 1 and 0.06 to 2 $\mu\text{L}/\text{mL}$, respectively, with *Candida glabrata* and *Candida dubliniensis* demonstrating the lowest MIC₅₀ and MIC₉₅ values.

According to Table 4, *Salvia mirzayani* essential oil at concentrations of 0.03 to 1 $\mu\text{L}/\text{mL}$ inhibited the growth of about half of the azole-sensitive *Candida*; in addition, MIC₅₀ value for the essential oil antifungal activity against azole-resistant *Candida* was 0.12 to 1 $\mu\text{L}/\text{mL}$.

Discussion

Results of this study showed monoterpenes 1,8-cineole, linalool acetate, and α -terpinyl acetate are the major constituents of *Salvia mirzayanii* essential oil. Our results are consistent with a previous study, which showed linalyl acetate (7.6%) and 1,8-cineole (8.0%) were 2 major volatile compounds of *Salvia mirzayanii*.¹⁴ However, Khoshnoud et al found that eudesm-7(11)-en-4-ol (15%) and 1,8-cineole were the 2 identified major compounds of *Salvia mirzayanii* essential oil.¹⁵ Furthermore, spathulenol (10.4%), δ -cadinene (5.8%), and linalool (5.2%) were dominant compounds in another report.¹⁶

According to a report by Zarshenas and Krenn, 12 studies have been carried out in the analysis of *Salvia mirzayanii* up to 2015. Major compounds were found to be α -terpinyl acetate,

linalyl acetate, 8-acetoxy linalool, spathulenol, linalool, 5-neo-cedranol, and eudesm-7(11)en-4-ol, while 1,8-cineole was reported in moderate amounts in 2 studies.¹⁷

Also, essential oil compounds were different in wild and cultivated *Salvia mirzayanii*. 5-Neo-cedranol (15.48%) and 1,8-cineol (11.23%) in wild type, and 5-neo-cedranol (19.42%) and spathulenol (7%) were the major components in cultivated *Salvia mirzayanii* essential oil.¹⁰

In other *Salvia* species, 1,8-cineol is the main constituents in their essential oil. This content is 17% in *Salvia multicaulis* in from Iran, 39.5% to 50.3% in *Salvia officinalis* of Jordan, and 12.4% to 17.6% in *Salvia officinalis* of Eastern Lithuania.¹⁸⁻²⁰ It seems that developmental stages, geographical region, environmental factors, genetics, as well as sampling are important factors to determine essential oil composition.^{7,21}

The terpene oxide 1,8-cineole (cineole, eucalyptol, cajepulol) was reported as major constituent of many essential oil such as *Eucalyptus globulus*, *Cinnamomum longepaniculatum*, *Rosmarinus officinalis*, *Psidium pohlianum*, *Psidium guyanensis*, and *Salvia libanotica*.^{22,23} These monoterpenes are not only considered in the pharmaceutical industry as drug ingredients (percutaneous penetration enhancer, decongestant, and antitussive agents) but also they possess broad application in medicinal treatment such as asthma, sinusitis, rheumatism, and septic-shock-associated pathologies.²³⁻²⁵ Antimicrobial, anticancer, anti-inflammatory, and antioxidation activities are further pharmacological effects of 1,8-cineole.²³

Candida species is the common cause of mucocutaneous infections and they are responsible for most bloodstream infections. Traditional triazole drugs' resistance of *Candida* species has been increased significantly for several years and thus the requirement of novel antifungals is considerable.²⁶ The results indicated essential oil of *Salvia mirzayanii* have strong anti-*Candida* activity with MIC₅₀ values 0.03 to 1 $\mu\text{L}/\text{mL}$ and 0.12 to 1 $\mu\text{L}/\text{mL}$ for candidia and azole-resistant candida, respectively.

These results are consistent with the study by Zomorodian et al, who reported strong anti-*Candida* activity of *Salvia mirzayanii* essential oil (MIC = 0.25-1 $\mu\text{L}/\text{mL}$).²⁷

Results of *Salvia mirzayanii* antifungal effect against the azole-resistant and azole-susceptible tested strains may be due to this fact that essential oils have different mechanisms of action in comparison to azole antifungal drugs. Cell membrane interaction is an important characteristic of hydrophobic essential oil and may be the main mechanism of this action.²⁸ Although the MIC value of essential oil on a panel of fungus is low, their application as antifungal therapeutic agents is restricted. Nevertheless, their lower side effects make them a source of novel natural antibiotics.²⁹

Staphylococcus aureus has been recognized as cause of life-threatening infections such as bacteremia. The incidence has been increased because of the rise in antibiotic-resistant strains, in particular, methicillin.³⁰

The growth of the standard and clinical isolates of methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus* was inhibited by *Salvia mirzayanii* essential oil at concentrations of 0.013 and 0.32 $\mu\text{L}/\text{mL}$, respectively. In a previous study, MIC for this

Table 2. Antibacterial Activity (MIC and MBC) of Essential Oil Distilled From *Salvia mirzayanii*.

Bacteria (Number of Strains)	MIC ₉₅ , GM μ L/mL (Range)	MBC, GM μ L/mL (Range)
Gram positive		
<i>Staphylococcus aureus</i> ATCC 25923	0.12	8
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) (5)	0.13 (0.03-0.25)	0.21 (0.12-4)
Methicillin-sensitive <i>Staphylococcus aureus</i> (MSSA) (6)	0.32 (0.12-0.5)	0.37 (0.25-0.5)
Vancomycin-resistant <i>Enterococcus faecalis</i> (4)	0.35 (0.25-0.5)	22.62 (16-32)
Vancomycin-sensitive <i>Enterococcus faecalis</i> (5)	0.37 (0.25-0.5)	22.62 (16-32)
Vancomycin resistant <i>Enterococcus faecium</i> (4)	0.35 (0.25-0.5)	22.62 (16-32)
<i>Streptococcus mutans</i> ATCC 35668	0.06	1
<i>Streptococcus pneumonia</i> ATCC 33400	>0.031	0.062
<i>Streptococcus pyogenes</i> ATCC 8668	>0.031	>0.031
Gram negative		
<i>Escherichia coli</i> 0157	64	>128
<i>Escherichia coli</i> ATCC 25922	16	>128
Third-generation cephalosporin-resistant <i>Escherichia coli</i> (6)	64	\geq 128
Third-generation cephalosporin-sensitive <i>Escherichia coli</i> (5)	97.00 (64-128)	64 \geq 128
Multidrug-resistant <i>Pseudomonas aeruginosa</i> (6)	>128	>128
Sensitive strain <i>Pseudomonas aeruginosa</i> (5)	>128	>128
<i>Shigella flexneri</i> NCTC 8516	16	32
<i>Salmonella enterica</i> ATCC 14028	>128	>128

Abbreviations: GM, geometric mean; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

essential oil to inhibit the growth of *Staphylococcus aureus* was 2.5 ± 0.3 and for *Staphylococcus hydrangea* and *Staphylococcus santolinifolia* it was $15 \mu\text{L}/\text{mL}$.⁹ Thus, it seems that our samples are more effective than previous samples. The proper effects of this essential oil on these resistant bacteria may be related to different antibacterial mechanisms of essential oils in comparison to β -lactam antibiotics.³¹

Enterococci are responsible for different infections such as urinary tract infections, bacteremia, and endocarditis. They are resistant to a number of antimicrobial drugs like vancomycin and some species (eg, *Enterococcus faecium*) are more resistant than others species. Vancomycin-resistant enterococci should be considered as significant resistant opportunistic pathogens in the hospital environment.³²

Antimicrobial activities of *Salvia mirzayanii* essential oil were strong against vancomycin-resistant *Enterococcus faecium* as well as both vancomycin-resistant *Enterococcus faecalis* and vancomycin-sensitive *Enterococcus faecalis*. Earlier finding showed MIC for *Salvia mirzayanii* essential oil to inhibit the growth of *Enterococcus faecalis* was $10 \mu\text{L}/\text{mL}$. (This was collected from Darab, Fars, rich in α -terpinenyl acetate and 1,8-cineol.) This value (MIC) was $\geq 15 \mu\text{L}/\text{mL}$ in other *Salvia* species.⁹ These evidences suggest that *Salvia mirzayanii* essential oils are more powerful against these bacteria in comparison to other reported species.

Among gram-positive bacteria, *Streptococcus pneumonia* and *Streptococcus pyogenes* seem to be the most sensitive to the essential oil. According to our finding, essential oil showed antibactericidal effects against both third-generation cephalosporin-resistant *Escherichia coli* (MIC₉₅ = $64 \mu\text{L}/\text{mL}$) and third-generation cephalosporin-sensitive *Escherichia coli* (MIC₉₅ = $97 \mu\text{L}/\text{mL}$). Our finding differ from previous results; for instance, Sonboli et al reported MIC $2.5 \text{ mg}/\text{mL}$ for *Escherichia coli* and $>15 \text{ mg}/\text{mL}$ for other *Salvia* species,⁹ and

Javidnia et al showed antibacterial activity of *Salvia mirzayanii* essential oil against *Escherichia coli* with MIC = $1 \text{ mg}/\text{mL}$ and MIC 0.25 to $2 \text{ mg}/\text{mL}$ for other *Salvia* species.³³

Our study suggested that the MIC₉₅ for *Pseudomonas aeruginosa* was $>128 \mu\text{L}/\text{mL}$, but in other studies the reported MIC was $20 \text{ mg}/\text{mL}$ ⁹ and $0.25 \text{ mg}/\text{mL}$, and this value was 0.5 to $1 \text{ mg}/\text{mL}$ for other *Salvia* species.³³ The importance of this finding is related to the role of *Pseudomonas aeruginosa* in progressive noteworthy hospital infections. Also, it possesses inherent multifactorial resistance to several antimicrobials by different mechanisms such as bacterial outer-membrane barrier, multidrug efflux transporters, and endogenous antimicrobial inactivation. Consequently, screening for novel antibacterial against *Pseudomonas aeruginosa* is currently considerable.^{33,34}

It seems that the MICs and MBCs of the essential oil against the examined gram-negative bacteria were higher than gram-positive bacteria. This evidence was similar to results of other studies.^{31,35-38} It seems that resistance of gram-negative bacteria to antimicrobials could be related to structure and composition of their cell envelope. Presence of peptidoglycan layer, phospholipids, and lipopolysaccharides in outer membrane make the cell wall of gram-negative bacteria more complex. This lipopolysaccharide coverage limits diffusion of hydrophobic compounds through this barrier. However, without this obstruction, the membrane permeation is more easily in gram-positive bacteria.^{1,36,39,40}

According to previous studies, the predominant antimicrobial activity of essential oil are the cell wall and cell membranes damages follow by cell lysis and leakage of cell content such as K^+ . Antimicrobial functions of essential oil severely depend on their chemical composition. Essential oils with hydrophobic characteristic penetrate microbial membrane, interfere in constitution of cell wall/membranes, and

Table 3. Antifungal Activity (MIC and MFC) of Essential Oil From *Salvia mirzayanii*.

Code	Species	<i>Salvia mirzayanii</i>			Fluconazol			Itraconazol			
		MIC ₅₀ (μ L/mL)	MIC ₉₅ (μ L/mL)	MFC (μ L/mL)	MIC ₅₀ (μ L/mL)	MIC ₉₅ (μ L/mL)	MFC (μ L/mL)	MIC ₅₀ (μ L/mL)	MIC ₉₅ (μ L/mL)	MFC (μ L/mL)	
1	St	<i>C.albicans</i>	0.5	1	2	2	G	G	0.03	G	G
2	St	<i>C.tropicalis</i>	0.12	0.25	1	16	32	G	0.5	G	G
3	St	<i>C.parapsilosis</i>	0.5	1	4	1	2	16	<0.03	0.03	0.25
4	St	<i>C.krusei</i>	0.25	1	4	8	16	G	<0.03	0.03	0.06
5	St	<i>C.glabrata</i>	0.12	0.25	0.5	2	4	G	0.03	0.06	G
6	St7	<i>C.glabrata</i>	0.03	0.06	2	2	4	G	0.25	0.5	G
7	St8	<i>C.dubliniensis</i>	0.12	0.5	2	0.5	G	G	G	G	G
8	St9	<i>C.dubliniensis</i>	0.03	0.06	0.5	G	G	G	0.25	G	G
9	St11	<i>C.albicans</i>	0.25	1	4	4	G	G	G	G	G
10	St12	<i>C.glabrata</i>	0.5	1	4	1	2	G	0.5	G	G
11	St13	<i>C.albicans</i>	0.25	1	4	0.12	1	64	0.03	G	G
12	St14	<i>C.albicans</i>	1	2	4	0.25	G	G	0.25	G	G
13	St15	<i>C.albicans</i>	0.25	0.5	1	0.25	0.5	G	0.03	0.06	16
14	St16	<i>C.albicans</i>	0.5	2	4	4	8	G	0.25	0.5	8
15	St17	<i>C.glabrata</i>	0.12	0.25	8	2	4	G	0.25	0.5	G
16	St18	<i>C.glabrata</i>	0.5	1	4	2	4	G	0.5	1	G
17	613	<i>C.parapsilosis</i>	0.25	1	4	0.12	0.25	0.5	0.06	0.12	0.25
18	194	<i>C.tropicalis</i>	0.5	2	4	G	G	G	G	G	G
19	203	<i>C.albicans</i>	0.5	2	4	4	G	G	G	G	G
20	611	<i>C.tropicalis</i>	1	2	4	G	G	G	G	G	G
21	617	<i>Trichosporon</i>	0.5	1	1	G	G	G	2	4	4
22	625	<i>C.albicans</i>	0.5	2	4	G	G	G	G	G	G
23	634	<i>C.albicans</i>	1	2	4	64	G	G	G	G	G
24	2303	<i>C.albicans</i>	1	2	4	G	G	G	G	G	G
25	2353	<i>C.albicans</i>	0.5	2	8	G	G	G	G	G	G
26	3018	<i>C.albicans</i>	0.12	1	4	G	G	G	G	G	G
27	2413	<i>C.albicans</i>	1	2	8	<0.12	0.12	G	<0.03	0.03	G
28	2662B	<i>C.albicans</i>	0.5	1	4	0.12	0.25	G	0.03	0.06	G
29	174	<i>C.albicans</i>	0.5	2	8	0.12	0.25	G	<0.03	0.03	G
30	2959	<i>C.albicans</i>	0.5	1	8	0.12	0.25	G	<0.03	0.03	G
31	537	<i>C.albicans</i>	0.5	1	4	0.12	1	64	<0.03	0.06	2
32	2358	<i>C.parapsilosis</i>	0.5	1	8	0.25	1	G	0.12	0.25	1
33	3086	<i>C.albicans</i>	0.5	1	2	0.12	0.25	16	<0.03	0.03	8
34	2622B	<i>C.parapsilosis</i>	0.25	1	4	0.12	0.25	G	<0.03	0.03	G
35	590	<i>C.parapsilosis</i>	0.12	0.25	1	0.25	1	4	<0.03	0.03	0.03
36	2397	<i>C.parapsilosis</i>	0.5	1	4	<0.12	4	4	0.03	0.25	0.5
37	620	<i>C.albicans</i>	0.5	1	2	0.12	0.5	G	<0.03	0.03	G
38	20945	<i>C.albicans</i>	1	2	4	0.12	0.25	64	0.03	0.06	1
39	5444	<i>C.albicans</i>	1	2	4	0.12	0.25	G	0.06	0.25	4
40	2398	<i>C.albicans</i>	1	2	4	<0.12	0.12	G	0.03	0.06	1

Abbreviations: MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration.

Table 4. Antifungal Activity of Essential Oil of *Salvia mirzayanii* against Azole-Sensitive and Azole-Resistant *Candida*.

Species (Number)	MIC ₅₀ , GM μ L/mL (Range)	MIC ₉₅ , GM μ L/mL (Range)	MFC, GM μ L/mL (Range)	
Azole sensitive	<i>C albicans</i> (16)	0.54 (0.25-1)	1.29 (0.5-2)	3.66 (2-8)
	<i>C ubliniensis</i> (2)	0.06 (0.03-0.12)	0.17 (0.06-0.5)	1 (0.5-2)
	<i>C glabrata</i> (5)	0.16 (0.03-0.5)	0.32 (0.06-1)	2.63 (1-2)
	<i>C krusei</i> (1)	0.25	1	4
	<i>C parapsilosis</i> (6)	0.31 (0.12-0.5)	0.79 (0.25-1)	3.56 (1-8)
	<i>C tropicalis</i> (1)	0.12	0.25	1
	Azole resistant	<i>C albicans</i> (6)	0.49 (0.12-1)	1.78 (1-2)
<i>C tropicalis</i> (2)		0.7 (0.5-1)	2	4

Abbreviations: GM, geometric mean; MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration.

destruct cellular activities.^{1,39} In gram-positive bacteria, hydrophobicity of bacterial and cytoplasmic membrane is affected by essential oil, while in gram-negative bacteria they are unable to penetrate bacterial cell wall.³⁹ Some other accepted mechanisms of essential oil antimicrobial effects are inhibition of biochemical pathway, inhibition of protective enzymes, and enhancement of uptake of other antimicrobials.⁴¹ Beside these general antimicrobial mechanisms of essential oil, monoterpenes increase membrane fluidity and permeability, changing the topology of membrane proteins and disturbing the respiration chain.⁴¹ It seems that 1,8-cineole, linalool acetate, and α -terpinyl acetate in essential oil of *Salvia mirzayanii* are responsible for antimicrobial activities of this essential oil.

Conclusion

Our results indicate that essential oil of *Salvia mirzayanii* possesses proper broad spectrum of antibacterial effects and antifungal activities. So this essential oil may be applied in design of novel antimicrobial agents according to these effects. Furthermore, nowadays rise of microbe resistance are considerable and natural products could be appropriate candidates to overcome this problem.

More researches are needed to determine the active therapeutic compounds and their mechanisms of action. Also, in vivo potential of these essential oils alone or in combination with current antimicrobial agents in animal models could be investigated in future studies.

Author Contributions

All authors contributed to the design, execution, analysis, and writing of this article.

Declaration of Conflicting Interests

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Ethical Approval

This work is an experimental and laboratorial study and needs no ethical approval.

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