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Antimicrobial activity of *Zhumeria majdae* Rech.F. & Wendelbo essential oil against different microorganisms from Iran

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ABSTRACT

The monotypic *Zhumeria majdae* Rech.F. & Wendelbo was recently described as the first member of new genus *Zhumeria* (*Lamiaceae*). In Iranian folk medicine, the leaves of plant were used for antiseptic properties. The antimicrobial activity of *Zhumeria majdae* essential oil was evaluated against a panel of microorganisms including gram negative, gram positive, yeast and fungi using disc diffusion method and micro broth dilution assay. Generally, the oil exhibited similar levels of antimicrobial activity against different microorganisms but some microorganisms appear to be more sensitive. In particular, oil showed significant power against *Klebsiella pneumoniae* followed by *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Vibrio cholera*, *Staphylococcus epidermidis* and *Bacillus cereus*. *Klebsiella pneumonia*e with MIC and MBC values 0.5, 1 µl ml^{-1} was the most sensitive among the tested microorganisms. The oil showed bactericidal activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli and Enterobacter aerugenes*. The oil showed inhibitory effect against *Bacillus subtilis*, *Proteus vulgaris*, *Aspergillus flavus* and *Aspergillus niger*. *Salmonella typhi* and *Pseudomonas aeruginosa* among Gram negative bacteria and *Bacillus subtilis* among Gram positive ones and *Aspergillus niger* among fungi and yeast were the most resistant to the essential oil.

KEYWORDS: Linalool, camphor, Mohrekhosh, antimicrobial activity

INTRODUCTION

The antimicrobial of essential oils have been recognized for many years and have been used as flavouring agents in food and beverages and due to the existence of antimicrobial compounds, they have a potential as natural agents in the field of pharmacology, pharmaceutical botany, Phytopathology, medical and clinical microbiology and food preservations (1, 2). *Zhumeria majdae* Rech. F. & Wendelbo (*Lamiaceae*) known locally by the name of "Mohrekhosh" is a monotypic genus of *Zhumeria* occurring in the southern parts of Iran (3) have been credited with a high potential medicinal uses as antinociceptive, anti inflammatory (4), antileishmanial, antiplasmodial (5). Its leaves have been used for many years as a curative for stomachaches, flatulence, diarrhea, indigestion, cold, headache, wound healing and as antiseptic and treatment of painful menstruation (6). Javidnia et al (7) has been analyzed the chemical composition of *Zhumeria majdae* essential oil from root and aerial part of this plant and found that Manool (37.1%) and O- Cedrol (6.0%) were the main constituents of root essential oil while linalool and camphor were the main compounds of aerial part of plant. There is only one report on antibacterial activity of its aerial part essential oil against *Escherichia coli* and *Staphylococcus aureus* using dilution method (8).This study

showed *E. coli* was sensitive to the oil than *S.aureus*. The aim of this study was to evaluate the antimicrobial activity of essential oil that extracted from aerial part of *Zhumeria majdae* against different microorganisms including gram positive, gram negative bacteria, fungi and yeast under laboratory condition.

METHODS AND MATERIAL

Z. majdae essential oil

Essential oil and its major components *Z. majdae of* known composition [linalool (54%), camphor (29%)] was obtained from Barij Essence Pharmaceutical Company, Kashan, Iran.

Microbial strains

All experiments were tested against a panel of microorganisms, including Gram-positive cocci *Staphylococcus aureus* ATCC 25923, *Staphylococcus saprophyticus* ATCC 13518, *Staphylococcus epidermidis* ATCC 12228, Gram positive spore bacilli *Bacillus subtilis* ATCC 6051, *Bacillus cereus* ATCC 1247 *and* non-spore bacilli *Listeria monocytogenes* ATCC 7644, Gram negative bacilli *Escherichia coli* ATCC 8739, *Salmonella typhi* ATCC 14028, *Shigella dysantri* RI 366, *Shigella flexeneri* (Clinical isolate), *Klebsiella pneumoniae* ATCC 10031, *Proteus vulgaris* RI 231, *Enterobacter aerugenes* NCTC 10009, *Vibrio cholera* Inaba, *Pseudomonas aeruginosa* ATCC 9027, Fungi *Aspergillus niger* ATCC 16404, *Aspergillus flavus* (Field isolate), yeast *Candida albicans* ATCC 10231. *Bacterial* strains were cultured overnight at 37°C on Nutrient agar. Yeast and fungi were cultured at 30°C on sabouraud dextrose agar.

Antimicrobial screening

Antimicrobial activity of the essential oil was determined, using disc diffusion method and micro broth dilution assays. The bacterium inocula were prepared by suspending overnight colonies from nutrient agar media in sterile normal saline. The *Candida albicans* and fungi inocula were prepared by suspending colonies from 48 and 72 h sabouraud dextrose agar cultures in RPMI 1640 medium (Sigma-Aldrich chemie GmbH, Steinheim, Germany) buffered with 0.165 M Morpholinepropanesulfonilic acid (MOPS) (Merck KGaA, Darmstadt, Germany). This inoculate was adjusted to 0.5 McFarland. The inoculum was 1×10^8 CFU ml^{-1} for bacteria and 1×10^7 CFU ml^{-1} for yeast and fungi. Suspensions of bacteria and fungi using a sterile cotton swab were cultured individually on Muller Hinton Agar and sabouraud dextrose Agar respectively. Subsequently, sterile discs (6 mm in diameter) (Padtan Teb Co, Tehran, Iran) was saturated with 2.5 µl of oil that dissolved in 18.5 µl of Dimethylsulfoxide (DMSO). Disc contains DMSO was used as control. The cultured plates were incubated at 37°C for 24 and 48 hours for bacteria and fungi respectively. The inhibition zone was diametered in millimeters and recorded (9). The minimal inhibitory concentration (MIC) values of essential oil against different microorganism were determined by micro broth dilution assay. The oil was twofold serially diluted with 10% DMSO which contains 8-0.0125 µl ml^{-1} of oil. RPMI 1640 with L-glutamine but lacking bicarbonate buffered with 0.165 M Morpholinepropanesulfonilic acids (MOPS) was used as broth media for fungi and yeast (10). Cation adjusted Muller Hinton broth was used as a broth medium for bacteria (11). After shaking, 100µl of the essential oil was added to each well. The suspension of each organism was adjusted to $1\times10^5-1\times10^6$CFU ml^{-1} and then 100 µl was added to each well and cultivated at 35°C. MIC values were defined as the lowest concentration of oil that inhibit of bacteria and fungi after 24, 48h respectively. Minimal bactericidal concentration (MBC) values were the first tube that showing no growth on solid media.

RESULTS

For evaluating, the antimicrobial activity of *Z. majdae* oil against different microorganisms, we used disc diffusion method and dilution assay for qualitative and quantitative screening of action of the oil *in-vitro*. In disc diffusion method, the inhibition zone diameter of 2.5 µl of *Z. majdae* oil is smaller than the inhibition zone of different antibiotics (Table1–3). The potency of essential oil was quantitatively assessed by determining the MIC and MBC as given in each table. The oil showed bactericidal activity against *S. aureus* (1, 1 µl ml^{-1}), *B. cereus* (2, 2 µl ml^{-1}) (Table1), *E. coli*, *E. aerugenes* (MIC and MBC values 4 µl ml^{-1}) (table 2). Among different microorganisms, *K. pneumoniae* is the most sensitive microorganism with MIC and MBC values 0.5, 1 µl ml^{-1} (Table2) following by *S. aureus* (1, 1 µl ml^{-1}), *S. saprophyticus*, *V. cholera* (1, 2 µl ml^{-1}) and *B. cereus*, *S. epidermidis* (2, 2 µl ml^{-1}). The oil showed inhibitory effect against *B. subtilis* (Table1), *P. vulgaris* (Table2), *A. flavus* and *A. niger* (Table3). The MBC values of previous microorganisms were multifold of their MIC values. No clear correlation between MIC values and inhibition diameters was found. Some gram negative bacteria had greater inhibition zone including *E. coli*, *S. typhi* than gram positive bacteria like *S. aureus*, *B. cereus* but the MIC and MBC values of these bacteria

Table 1: Antimicrobial activity of *Z. majda*e oil against gram positive bacteria

organisms	Inhibition Zone diameter(mm)		Minimal concentration values(µl ml⁻¹)	
	essential oil	Antibiotics	MIC	MBC
		Van		
S. aureus	11	17	1	1
S. epidermidis	8	19	2	2
S. saprophyticus	10	19	1	2
		Ery		
L. monocytogenes	11	36	2	4
B. subtilis	13	29	1	8
B. cereus	11	26	2	2

Van= Vancomycin, Ery= Erythromycin

Table 2: Antimicrobial activity of *Z. majdae* oil against gram negative bacteria

organisms	Inhibition Zone diameter(mm)		Minimal concentration values(µl ml⁻¹)	
	essential oil	Gentamycin	MIC	MBC
E. coli	19	20	4	4
P. aeruginosa	10	23	4	8
S. typhi	15	19	4	8
Sh. dysantri	11	12	2	4
Sh. flexeneri	12	18	2	4
K. pneumoniae	16	21	0.5	1
P. vulgaris	8	22	1	4
E. aerugenes	–	19	4	4
V. cholera	13	28	1	2

Table 3: Antimicrobial activity of *Z. majdae* oil against yeast and fungi

organisms	Inhibition Zone diameter(mm)		Minimal concentration values(µl ml⁻¹)	
	essential oil	Amphotricin B	MIC	MBC
A. niger	_	14	0.5	8
A. flavus	_	10	2	8
C. albicans	10	17	2	4

were larger of them. Fungi were less sensitive to the oil than *C. albicans*.

DISCUSSION

The increasing antibiotic resistance of some pathogens that caused infectious diseases as well as the appearance of undesirable side effects of antibiotics has increased the interest of new nontoxic and more effective antimicrobial agents among herbal plants. The use of plant compounds to treat infections is an age old practice in a large part of countries. Among the medicinal or aromatic plants, the antimicrobial activity of *Z. majdae* has not been studied very well. In Iranian folk medicine, the leaves of plant used for treatment of wound healing. The aerial part of this species produces the essential oil rich in linalool and camphor. Linalool (Tertiary alcohol) is active against the microorganisms potentially acting as either a protein denaturing or as solvent dehydrating agent (12). Essential oil always represents complex mixtures of compounds. The nature and proportion of individual constituents of oil could influence on their antimicrobial activity, for that reason, it is very difficult to reduce the antimicrobial activity of *Z. majdae* oil contains linalool to one or a few active principle. No clear correlation between MIC values and inhibition diameters was found, suggesting that the results gained with these two methods are not necessarily comparable. Absorption essential oil in liquid media is determined by solubility, volatility and stability of volatile compounds. In conclusions, the antimicrobial activity of *Z. majdae* oil against different microorganisms varies widely depending on the type of microorganisms; test medium and its antimicrobial activity were germicidal or inhibitory against different microorganisms. The effect of oil on spores differs than germination cells. Gram negative bacteria were shown to be generally more resistant than gram positive ones because of the lipopolysaccharide present in outer membrane (13), but this was not always true.

Therefore, the results of this study seem to be promising and may enhance the natural product uses, showing the potential of *Zhumeria majdae* oil in the treatment of infections caused by some microorganisms especially *K. pneumoniae*. Further studies should be carried out for antimicrobial activity of oil on clinical isolates of *K. pneumoniae in vitro* and *in vivo*.

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