

Composition and antifungal activity of *Zhumeria majdae* essential oil

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(Received: 7 November 2015; Revised: 12 December 2015; Accepted: 16 December 2015)

Abstract

Background and Purpose: Essential oils extracted from different plants are extensively used in perfume, beverage, and food industries and are reported to exhibit antimicrobial activities against a variety of fungi. *Zhumeria majdae* belonging to the *Lamiaceae* family is a rare and endemic medicinal plant species in Iran, with a strong and pleasant odor. The leaves of this plant have been used for many years as an antiseptic carminative agent for the treatment of stomachache (especially in infants) and dysmenorrhea.

Materials and Methods: Gas chromatography/mass spectrometry (GC/MS) analysis was performed to determine the main constituents of the essential oil extracted from the aerial parts of *Z. majdae*. Also, the minimum inhibitory concentrations (MICs) were determined, using serial dilution method.

Results: Based on the GC/MS analysis, 31 compounds representing 95.36% of the essential oil, extracted from the aerial parts of the plant, were identified, among which linalool (63.40%) and camphor (27.48%) were recognized as the major constituents. The total phenolic content was 42.74 GAE (mg)/DW (g). The hydro-distilled essential oil from the aerial part of the plant displayed potential antifungal activities against all the tested pathogenic fungal species (i.e., *Candida albicans*, *Trichophyton mentagrophytes*, *Aspergillus flavus*, *Trichophyton rubrum*, *Microsporum canis*, *Microsporum gypseum*, and *Epidermophyton floccosum*). Based on the inhibition zone (29 mm) and MIC value (0.015 µl/ml), all the tested strains were sensitive to *Z. majdae* essential oil.

Conclusion: The present results support the traditional and possible use of *Z. majdae* essential oil in food, pharmaceutical, and cosmetic industries.

Keywords: Antifungal, Chemical composition, Minimum inhibitory concentration, Plant Extracts

➤ How to cite this paper:

Imani Z, Asgarpanah J, Hashemi F, Hashemi Hezaveh J. Composition and antifungal activity of *Zhumeria majdae* essential oil. *Curr Med Mycol*. 2015; 1(4): 13-19. DOI: [10.18869/acadpub.cmm.1.4.13](https://doi.org/10.18869/acadpub.cmm.1.4.13)

Introduction

Despite modern advances in slaughter hygiene and food production techniques, food safety has become an increasingly important public health concern. Nearly 30% of the population in industrialized countries is estimated to suffer from a food-borne disease each year. In 2000, at least two million people died from diarrhoeal diseases, worldwide [1, 2].

In recent years, use of natural substances has gained considerable attention in medical communities, and further research on plant resources has been encouraged to answer questions regarding the safety of synthetic compounds. Essential oils, which are odorous and volatile products of plant secondary metabolism, are extensively applied in traditional medicine, food flavoring and preservation, and fragrance industries [3].

Essential oils, also called volatile or ethereal oils [4], are composed of lipophilic and highly volatile secondary plant metabolites, reaching a mass below a molecular weight of 300 g/mol. Essential oils can be separated from other plant components or membrane tissues. The International Organization for Standardization (ISO 9235, 1997) has defined essential oil as "a product obtained from vegetable raw material, either by distillation with water or steam, or from the epicarp of *Citrus* fruits by a mechanical process, or by dry distillation" [5].

So far, nearly 3000 types of essential oils have been recognized, 300 of which are of commercial importance, particularly for pharmaceutical, agronomic, food, sanitary, cosmetic, and perfume industries [6]. Plants

belonging to the *Lamiaceae* family have been more frequently used as flavoring or medicinal agents, considering the significant amount of the extracted essential oils, compared to other medicinal plants [7].

Zhumeria majdae, which is locally known as “Mehrkhosh” in Hormozgan Province, grows in southeastern Iran. This plant with a strong and pleasant odor belongs to the *Lamiaceae* family [8, 9]. The use of this plant for stomachache and dysmenorrhea has been reported in traditional medicine [10] and its anti-nociceptive and anti-inflammatory activities have been recognized by researchers [8].

Z. majdae is used for the treatment of various disorders including diarrhea, cold, acid reflux, and headache and is applied as a carminative for wound healing [11, 12]. The present study aimed to describe the chemical composition, total phenolic content, and antifungal activity of essential oils extracted from the aerial parts of *Z. majdae*.

Material and Methods

Plant materials

The aerial parts of *Z. majdae* were harvested in the flowering stage from the natural habitat of this plant under artificial plant growth conditions. The harvested plants were dried at room temperature (25°C) for two weeks. Then, the air-dried plants from each habitat were ground and powdered with a mixer for essential oil extraction and other experiments.

Preparation of the extracts

The plant powder was extracted via hydro-distillation for 3 h, using a Clevenger apparatus. For the infusion, one liter of hot water was added to 100 g of the plant material, boiled for 15 min, and filtered through a cloth.

Preparation of the test samples

The essential oil (100 µl) was diluted with sterile distilled water to prepare a 5 ml stock solution, which was further diluted to prepare the test samples.

Gas chromatography/mass spectrometry (GC/MS) and GC analyses

The analysis of essential oils was carried out,

using GC and GC/MS methods. The GC apparatus was Agilent Technology (Model 6890USA, HP) with an HP-5MS capillary column (60 m×0.25 mm, film thickness of 0.25 µm). The oven temperature was initially set at 40°C for 1 min and then raised up to 230°C for 10 min (at a rate of 3°C per min).

Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The detector and injector temperatures were set at 250°C and 230°C, respectively. The GC/MS analysis was conducted on the GC system (Model 6890, HP), coupled with a 5973 Network Mass Selective Detector and a capillary column (HP-5MS capillary column).

Total phenolic content

The total phenolic content of *Z. majdae* essential oil was specified, using the Folin-Ciocalteu reagent and the method proposed by Spanos and Wrolstad (1990), which was later revised by Lister and Wilson (2001) [13, 14]. Afterwards, 2.5 ml dilution (1:10) of Folin-Ciocalteu reagent and 2 ml of Na₂CO₃ (7.5%, w/v) were added to 50 µl of each sample (three replicates) and incubated at 45°C for 15 min. The absorbance of all the samples was measured at 765 nm, using a SpectraMax-Plus 384 ultraviolet–visible spectrophotometer. The values were expressed as gallic acid equivalent (GAE) in milligram per gram of dry weight (DW).

Fungal strains

For the bioassays, seven species of different fungi were used: *Candida albicans* (ATCC 10231), *Trichophyton mentagrophytes* (ATCC 9533), *Aspergillus flavus* (ATCC 204304), *Trichophyton rubrum* (ATCC 28188), *Microsporum canis* (ATCC 36299), *Microsporum gypseum* (ATCC 24102), and *Epidermophyton floccosum* (ATCC 15694).

Serial dilution method

Antifungal activities of essential oils, prepared in a diluent containing dimethyl sulfoxide (DMSO), were determined, using the serial dilution method. For adequate growth, all the strains were grown on Sabouraud’s dextrose broth (SDB; Sigma-Aldrich, USA). The aqueous essential oil was

mixed with the liquefied agar medium at 45–50°C, poured in a microtube, and left to solidify. Moreover, a microtube series was prepared through increasing the concentration of the essential oil.

By using the applicator, different strains were inoculated on each plate. Following overnight incubation, the minimum inhibitory concentration (MIC) endpoint was calculated by placing the microtube against a dark background and determining the lowest concentration of derivatives impeding visible growth; the MIC of essential oil was recorded in µg/ml. The test was repeated in case two or more colonies persisted beyond the determined endpoint or if growth was observed at a higher concentration (not a lower concentration).

The punch well/cup plate diffusion method

In this technique, the melted agar, inoculated with a variety of microorganisms, was poured in Petri dishes of the agar medium. After the agar settled, cups were placed in the agar Petri dishes. Based on the results obtained by serial dilution method, *Z. majdae* essential oil solutions were prepared at the following concentrations: 0.007, 0.015, 0.031, 0.062, 0.125, 0.25, 0.5, 1, 2, and 4 µg/ml, respectively.

Statistical analysis

All experimental measurements were carried out in triplicate and the values were expressed as the average of three analyses (\pm standard deviation). The correlation between variables was assessed, using SPSS version 19 (Chicago, IL, USA).

Results

Essential oil composition

The GC/MS analysis of essential oils led to the identification of 17 different organic compounds, representing 99.13% of the total content of oils, extracted from the aerial parts of *Z. majdae*. The identified chemical compounds are listed in Table 1, according to their elution order on the capillary column. The essential oils mainly contained a complex mixture of oxygenated mono- and sesquiterpene hydrocarbons.

In this study, the major organic compounds detected in the aerial parts of *Z. majdae* essential oil were linalool (63.4%), camphor (27.48%), trans-linalool oxide (1.11%), limonene (0.98%), geraniol (0.9%), borneol (0.83%), trans-linalool oxide (0.81%), p-menth-1-en-8-ol (0.63%), camphene (0.56%), 3-octanone (0.43%), caryophyllene oxide (0.40%), benzene, 1-methyl-3-(1-methylethyl) (0.39%), Z-citral (0.32%), terpinen-4-ol (0.31%), geranial (0.28%), α -pinene (0.16%), and trans-caryophyllene (0.14%).

Total phenolic content and antioxidant activity

The total phenolic content of *Z. majdae* essential oil was measured by Folin-Ciocalteu reagent and expressed as GAE (standard curve equation: $y=0.04812x+0.0452$, $R^2=0.9901$; data not shown). The total phenolic content of compounds in the extracts was 42.74 GAE (mg)/DW (g).

Antifungal activity

The antifungal activities of *Z. majdae* essential oil are presented in tables 2 and 3. The antifungal

Table 1. Percentage of *Z. majdae* essential oil components

Compounds (total percentage)	
α -pinene	0.16 \pm 0.05
Camphene	0.56 \pm 0.02
3-Octanone	0.43 \pm 0.04
Benzene, 1-methyl-3-(1-methylethyl)	0.39 \pm 0.02
Limonene	0.98 \pm 0.01
Cis-linalool oxide	1.11 \pm 0.09
Trans-linalool oxide	0.81 \pm 0.01
Linalool	63.40 \pm 3.18
Camphor	27.48 \pm 1.16
Borneol	0.83 \pm 0.02
Terpinen-4-ol	0.31 \pm 0.02
P-Menth-1-en-8-ol	0.63 \pm 0.04
Z-citral	0.32 \pm 0.05
Geraniol	0.90 \pm 0.07
Geranial	0.28 \pm 0.01
Trans-caryophyllene	0.14 \pm 0.01
Caryophyllene oxide	0.40 \pm 0.02
Oil yield (% w/w)	4.02
Total	99.13

*Each value in the table was obtained by calculating the average of three experiments (\pm standard deviation).

**Data are expressed as the total percentage

Table 2. Results of serial dilution assay of *Z. majdae* essential oil

Fungal strains	Concentrations of <i>Z. majdae</i> essential oil ($\mu\text{l/ml}$)									
	0.007	0.015	0.031	0.062	0.125	0.25	0.5	1	2	4
<i>Candida albicans</i>	-	-	+	+	+	+	+	+	+	+
<i>Trichophyton mentagrophytes</i>	-	-	-	-	+	+	+	+	+	+
<i>Aspergillus flavus</i>	-	-	-	-	-	+	+	+	+	+
<i>Trichophyton rubrum</i>	-	-	-	-	+	+	+	+	+	+
<i>Microsporum canis</i>	-	-	-	-	+	+	+	+	+	+
<i>Microsporum gypseum</i>	-	-	-	-	+	+	+	+	+	+
<i>Epidermophyton floccosum</i>	-	-	-	-	+	+	+	+	+	+

(-) Represents no growth inhibition/resistance; (+) represents growth inhibition/susceptibility

activity was assessed by the measurement of inhibition zone, using a film disk containing the antifungal agent [15]. The results showed that the control films did not inhibit the growth of pathogenic fungal strains (n=7).

Z. majdae essential oil showed antifungal effects against all the studied fungal strains. In general, the essential oil showed significant inhibitory effects (7.84-29.05 mm). Based on the findings, the essential oil was most effective against *C. albicans* (inhibition zone diameter= 29.05 mm and MIC= 0.031 $\mu\text{l/ml}$), while *A. flavus* (inhibition zone diameter= 7.84 mm and MIC= 0.25 $\mu\text{l/ml}$) was the most resistant species. It should be noted that all the samples showed very strong antifungal activities.

Discussion

Essential oil composition

The present results showed that linalool and camphor are the main components of *Z. majdae* essential oil. Linalool (a terpene alcohol chemical) and camphor (a terpenoid) are naturally occurring compounds, which can be found in

Table 3. Punch well/cup plate diffusion results of *Z. majdae* essential oil

Fungal strains	Inhibition zone (IZ)
<i>Candida albicans</i>	29.05 \pm 0.03
<i>Trichophyton mentagrophytes</i>	15.22 \pm 0.07
<i>Aspergillus flavus</i>	7.84 \pm 0.04
<i>Trichophyton rubrum</i>	15.05 \pm 0.06
<i>Microsporum canis</i>	17.98 \pm 0.04
<i>Microsporum gypseum</i>	17.44 \pm 0.02
<i>Epidermophyton floccosum</i>	21.16 \pm 0.05

*Each value in the table was obtained by calculating the average of three experiments (\pm standard deviation).

various flowers and over 200 spice plants. Multiple commercial applications are attributed to these compounds, the majority of which are based on the induced pleasant odor [16-18].

In this study, the composition of *Z. majdae* essential oil was similar to that described by other researchers. According to a study by Rustaiyan (1992), *Z. majdae* essential oil mainly consists of monoterpenes (about 97 %). The ratio of linalool to camphor in this plant was nearly 1:1 in 1988, whereas in 1990, this ratio was reported to be approximately 2:1 [19].

Some sesquiterpenes (about 1%) occur only in traces of *Z. majdae* [19]. Based on a study by Javidnia et al. (2006), the yield of *Z. majdae* essential oil was 0.04% (w/w) and linalool and camphor, as the main compounds of the essential oil from the aerial parts of the plant, accounted for 2.1% and 0.8% of the stem oil, respectively [20].

In a study by Ebadollahi et al. (2014), GC/MS analysis of essential oil revealed that linalool (58.3%) and camphor (25.9%) are the main components of *Z. majdae* essential oil [21]. In a similar study by Majrouhi (2009), 22 components were identified in the essential oil of *Z. majdae* including 11 monoterpene hydrocarbons (13.8%), eight oxygenated monoterpenes (83.7%), and two sesquiterpenes (0.6%), representing nearly 99% of the total composition [22].

Total phenolic content and antioxidant activity

Antioxidants are able to reduce or prevent lipid oxidation in food products [23]. In fact, synthetic antioxidants have been extensively applied to impede lipid oxidation in foods [24].

However, use of such synthetic antioxidants is not preferred due to toxicological concerns. Based on previous findings, at a concentration of 50 mg/kg/day (500 times higher than the mentioned value), butylated hydroxyanisole and butylated hydroxytoluene seem to be free of any apparent adverse effects [25].

Based on the aforementioned background, there has been an increasing interest in identifying plant extracts to minimize or inhibit lipid oxidation in lipid-based food products [26]. Most of these natural antioxidants originate from fruits, vegetables, spices, grains, and herbs [27]; consequently, detection and application of more effective antioxidants are essential [28]. Antioxidant supplements may be used to help reduce oxidative damage in the human body [29]. Plants belonging to the *Lamiaceae* family have been used more frequently as antioxidant agents, compared to other plants, given the significant amount of the extracted essential oils [9].

According to a study by Moein and Moein (2010), the highest amount of phenolic compounds (1.98 ± 0.01 mg/g) was detected in ethyl acetate extracts of *Z. majdae* [12], while in a study by Sharififar et al. (2007), the total phenolic content was the highest in the methanolic extracts (50.1 ± 2.3 µg/mg) [9]. The variability in the total phenolic content in the conducted studies could be attributed to the changing solubility of phenolic compounds; also, the variation in solubility may be related to the solvent polarity [30, 31].

In some previous studies, methanol and ethanol were considered as more valuable solvents for phenolic extraction from plant materials, compared to less polar solvents such as acetone and hexane [31-33]. The present study suggested that *Z. majdae* has a high phenolic content and exhibits significant antioxidant activities. These findings suggest that the major part of antioxidant activities in *Z. majdae* results from the phenolic compounds.

Antifungal activity

An antifungal agent selectively eliminates fungal pathogens from the host while inducing minimal toxicity [34]. According to recent studies, the essential oils of various plants

belonging to the *Lamiaceae* family have a broad range of biological activities, notably antifungal potency, which is generally correlated with the chemical composition of the essential oil [35]. Overall, terpenes and flavonoids (natural phenols) constitute the active antifungal compounds of essential oils. It seems that the mechanism of antifungal or antibacterial action may be related to that of other compounds [36].

In the present study, *Z. Majdae* essential oil showed antifungal effects against all the tested fungi. Based on some earlier studies on the antibacterial properties of essential oils extracted from several species (from various genera), diverse degrees of growth inhibition were reported against some *Staphylococcus* and *Bacillus* species due to varying chemical compositions of the essential oils [37-39].

In a study by Burt (2004), *Z. majdae* essential oil, given its antifungal properties, could be potentially used in aromatherapy, pharmaceutical sciences, and pathogenic systems for the prevention of microbial growth. Therefore, *Z. majdae* could become an alternative to synthetic fungicides for application in agro-industries. Moreover, this plant could be used to screen and develop selective and natural fungicides for the treatment of many microorganisms, causing severe destruction of crops, vegetables, and ornamental plants.

Conclusion

The present results support the traditional and possible use of *Z. majdae* essential oil in food, pharmaceutical, and cosmetic industries.

Acknowledgments

The authors would like to thank Islamic Azad University (Pharmaceutical Sciences Branch, Faculty of Pharmacy, Department of Pharmacognosy) and Tehran University of Medical Sciences for their invaluable contribution. We also extend our gratitude to Leila Hosseinpoor for her technical assistance at School of Public Health.

Authors' Contributions

I. Z. performed all the tests and wrote the draft. A. J. and H. H. J. designed and managed

the research and edited the final manuscript. H. F. helped analyze the data.

Conflicts of interest

No potential conflicts of interest were reported in this study. All authors are responsible for the content and writing of the paper.

Financial disclosure

No financial interests related to the material of this manuscript were declared.

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