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Original research

Chemical composition, total phenolic content and antimicrobial activities of *Zhumeria majdae*

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ABSTRACT

Zhumeria majdae is a rare an endemic medicinal plant species grown wild in Iran. The hydro distilled essential aerial part oil of *Z. majdae* analysed by GC–MS. Thirty-one compounds representing 95.36% of the aerial part oil, respectively, were identified, of which linalool (48.14%), camphor (20.71%), limonene (4.83%), trans-linalool oxide (2.69%), 3-Octanone (2.34%), terpinene-4-ol (2.30%), camphene (1.88%), caryophyllene oxide (1.34%), geraniol (1.23%), α-pinene (1.22%), p-Cymene (1.08%) were the major compounds. Total phenolic content was 42.74 mg GAE/g dw and the IC_{50} in the radical scavenging assay was 32.08 mg/ml. The hydro distilled essential aerial part oil display potential of antibacterial activity against the tested 8 phytopathogenic bacterial such as *Bacillus cereus*, *Bacillus thuringiensis*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Salmonella typhimurium*, *Campylobacter jejuni*, *Klebsiella pneumoniae* and *Pseudomonas fluorescens*. The inhibition zones and MIC values for all test strains, which were sensitive to the essential oil of *Z. majdae* were in the range of 33-30 mm and 0.93 mg/ml, respectively. The results support the traditional usage and also possible use of *Z. majdae* essential oil and extracts in the food, pharmaceutical and cosmetic industries.

Keywords: Zhumeria majdae, Chemical composition, Total phenolic content, Inhibition Zone (IZ), Minimum inhibitory concentration (MIC)

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1. Introduction

The aberrant use of synthetic insecticides had several negative impacts on environment deliberately causing health hazards to humans ,bringing about resistant insect populations ,dis-rupting natural enemies in the biological control system and eventually leading to outbreaks of insect pests (Athie & Mills, 2005; Chowdhury et al., 2012).

The current trend is the search for and use of alternative methods to manage pests, which, in the economic context ,are effective without presenting the risks associated with the use of conventional Essential oils .Plants have acquired effective defence mechanisms that ensure their survival under adverse environmental conditions. In addition to morphological mechanisms, plants have also developed chemical defence mechanisms towards organisms such as insects that affect their biochemical and physiological functions (Prakash & Rao, 1996).

These chemicals are regarded as secondary plant metabolites. Essential oils derived from plants have the potential to play a major role in pest management in sustainable agricultural production. They are renewable, non-persistent in the environment and relatively safe to natural enemies, non-target organisms and human beings (Isman, 2006). Essential oils of herbs and their components, which are products from the secondary metabolism of plants, have many applications in ethno-medicine food, flavoring and preservation as well as in the fragrance and pharmaceuticals industries (Edris, 2007).

Plants belonging to the *Lamiaceae* family, due to high amount of essential oil, have been used more than other medicinal plants as flavoring agents or for medicinal uses. The volatile compounds have great application and demand in food, perfumery, cosmetics, pharmaceutical and winery industries (Jones & AbuMweis, 2009).

Zhumeria majdae, vernacular name “*Mehrkhosh*” ,grows in southeastern Iran. It has a strong and pleasant smell (Hosseinzadeh et al., 2002). Due to the reported use of this plant in folk medicine for stomachache and dysmenorrhea (Zargari, 1995) and reported on its antinociceptive and anti-inflammatory activities by other researchers (Hosseinzadeh et al., 2002).

The present research describes the results of our study on the chemical composition, total phenolic content and antimicrobial activity of the essential oils from *Zhumeria majdae* aerial part.

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2. Material and Methods

2.1. Plant materials

The aerial parts of *Zhumeria majdae* were harvested in flowering stages from natural habitat and artificial plant growth conditions. The harvested plants were dried at room temperature (25°C) for 2 weeks, then, air-dried plants (in each habitat) were ground and powdered with mixer for essential oil extraction and other experiments.

2.2. Essential oil extraction

The dried aerial parts of *Zhumeria majdae* in each habitat were subjected to hydro-distillation for 3 hours using a Clevenger-type apparatus. The essential oil obtained was separated from water and dried over anhydrous sodium sulfate and stored in sealed amber flasks at 4°C till analysis.

2.3. GC/GC-MS analysis

The oil analysis was carried out using GC and GC-MS. The GC apparatus was Agilent technology (HP) 6890 system, capillary column of HP-5MS (60 m × 0.25 mm, film thickness 0.25 µm). The oven temperature program was initiated at 40°C, held for 1 min then raised up to 230°C at a rate of 3°C /min held for 10 min.

Helium was used as the carrier gas at a flow rate 1.0 ml/min . The detector and injector temperatures were 250°C and 230°C respectively .GC-MS analysis was conducted on a HP 6890 GC system coupled with 5973 network mass selective detector with a capillary column the same as above, carrier gas helium with flow rate 1ml/min with a split ratio equal to 1/50, injector and oven temperature programmed was identical to GC.

2.4. Total phenolic content

Total phenolic content in *Zhumeria majdae* oil was determined by the Folin-Ciocalteau colorimetric method and determined as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g DW) using the standard curve.

2.5. Free radical scavenging capacity

Total The percent inhibition of DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical was calculated by the formula:

$$\text{Inhibition } (\%) = \left[\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right] \times 100 \qquad (1)$$

where, A_{blank} is the absorbance of the control reaction (DPPH alone), and A_{sample} is the absorbance of DPPH solution in the presence of the plant extract. IC_{50} values denote the concentration of the sample, required to scavenge 50% of DPPH free radicals.

2.6. Microbial strains

Eight microbial strains were used: *Bacillus cereus* (ATCC 14579), *Bacillus thuringiensis* (ATCC 10792), *Enterococcus faecalis* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12228), *Salmonella typhimurium* (ATCC 14028), *Campylobacter jejuni* (ATCC 33560), *Klebsiella pneumoniae* (ATCC 10031) and *Pseudomonas fluorescens* (ATCC 13525).

2.7. Determination of antimicrobial activity by the disk diffusion method

Antibacterial activity of aqueous extract was determined by disc diffusion method on nutrient agar medium. Whatmann filter discs (6 mm diameter) were made in nutrient agar plate using sterile cork borer and inoculums containing 53 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 100µl each of aqueous extract was placed in the discs made in inoculated plates .Plates were incubated for 24h at 37°C and zone of inhibition if any around the wells was measured in millimeter.

All treatment consists of three replicates and repeated at twice. The determination of minimum inhibitory concentration (MIC) as the lowest concentration of *Zhumeria majdae* extract inhibiting the growth of the organism, was determined based on the readings.

2.8. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of essential oil was determined by a twofold dilution method against *B. cereus*, *B. thuringiensis*, *E. faecalis*, *S. epidermidis*, *S. typhimurium*, *C. jejuni*, *K. pneumoniae* and *P. fluorescens*.

Parahaemolyticus Serial two-fold dilutions of the essential oil were made in Mueller-Hinton Broth containing 0.5% Tween 80 for bacteria in 96-well microtiter plates. Fresh microbial suspensions prepared from overnight grown cultures in the same media were added to give a final concentration of 5×105 organisms/ml. Controls of media with microorganisms or essential oil alone were included.

The microplates were incubated at 37ºC for 24 hours for bacteria. The first dilution with no microbial growth was recorded as MIC.

3. Results

3.1. Essential oil composition

The essential oils analysis by using GC–MS had led to the identification of 31 different organic compounds, representing 95.36% of the total oils from aerial part. The identified chemical compounds are listed in Table 1 according to their elution order on a capillary column. The essential oil contains a complex mixture consisting of mainly oxygenated mono and sesquiterpene hydrocarbons.

The major organic compounds detected in the aerial part oil were linalool (48.14%), camphor (20.71%), limonene (4.83%), trans-linalool oxide (2.69%), 3-Octanone (2.34%), terpinene-4-ol (2.30%), camphene (1.88%), caryophyllene oxide (1.34%), geraniol (1.23%), α-pinene (1.22%), p-Cymene (1.08%), Trans-ocimene (0.87%), Geranial (0.85%), Nerol (0.69%), Thymol (0.64%), α–Terpineol (0.60%), Cis- ocimene (0.58%), Borneol (0.58%), γ-Terpinene (0.43%), Neral (0.40%), α-Terpinene

(0.31%), 1, 8-Cineol (0.31%), p-Cymene-8-ol (0.29%), Terpinolene (0.22%), Piperitenone (0.22%), Cis-Jasmone (0.21%), (E)-Caryophyllene (0.14%), Carvacrol (0.13%), Myrcene (0.5%), Cis-linalool oxide (0.5%) and β-pinene (0.3%).

Mono- and sesquiterpene hydrocarbons were the characteristic constituents of the oils of *Zhumeria majdae*.

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

Compounds	Percentage of total
Linalool	48.14 ± 2.90
Camphor	20.71 ± 1.08
Limonene	4.83 ± 0.72
Trans-linalool oxide	2.69 ± 0.81
3-Octanone	2.34 ± 0.59
Terpinene-4-ol	2.30 ± 0.05
Camphene	1.88 ± 0.37
Caryophyllene oxide	1.34 ± 0.42
Geraniol	1.23 ± 0.51
α-pinene	1.22 ± 0.32
p-Cymene	1.08 ± 0.08
Trans- ocimene	0.87 ± 0.11
Geranial	0.85 ± 0.04
Nerol	0.69 ± 0.05
Thymol	0.64 ± 0.19
α -Terpineol	0.60 ± 0.10
Cis- ocimene	0.58 ± 0.07
Borneol	0.58 ± 0.11
γ-Terpinene	0.43 ± 0.09
Neral	0.40 ± 0.12
α-Terpinene	0.31 ± 0.10
1,8-Cineol	0.31 ± 0.05
p-Cymene-8-ol	0.29 ± 0.06
Terpinolene	0.22 ± 0.01
Piperitenone	0.22 ± 0.11
Cis-Jasmone	0.21 ± 0.03
(E)-Caryophyllene	0.14 ± 0.04
Carvacrol	0.13 ± 0.02
Myrcene	0.05 ± 0.01
Cis-linalool oxide	0.05 ± 0.01
β-pinene	0.03 ± 0.01
Oil Yield (%w/w)	4.02
Total	95.36

*Each value in the table was obtained by calculating the average of three experiments ± standard deviation.
**Data expressed as percentage of total.

3.2. *Total phenolic content and antioxidant activity*

Antioxidants can minimize or prevent lipid oxidation in food products (Shahidi et al., 1992). Synthetic antioxidants such astert butylhydroxytoluene, tert-butylhydroxyanisole, and tert-butylhydroquinone (TBHQ) have been widely used to retard lipid oxidation in foods (Ahmad, 1996).

However, such synthetic antioxidants are not preferred due to toxicological concerns. For this reason, there have been increasing interests in identifying plant extracts to minimize /retard lipid oxidation in lipid-based food products (Ahn et al., 1998). Most of these natural antioxidants come from fruits, vegetables, spices , grains, and herbs (Buckley et al., 1995).

Zhumeria majdae is a unique plant that belongs to *Labiatae* family. Analysis of essential oil showed that major constituents are camphor and linalool (Majrouhi, 2009). The total phenolic content in *Zhumeria majdae essential* oil were measured by Folin Ciocalteau reagent and expressed as gallic acid equivalent (standard curve equation: y = 0.04812x + 0.0452, R^2 = 0.9901 data not shown).

The highest total phenolic content was 42.74 mg GAE/g DW. The antioxidant activity of the methanolic extracts of the aerial parts of *Z. majdae* in wild and cultivated conditions were assessed by the DPPH free radical scavenging method. The highest antioxidant activity was 32.08 μg/ml). Anyway, the correlation between antioxidant activities of plant extracts and their individual phenolic compounds can be used as a clue for prevention of oxidation (Rababah et al., 2004). In fact, with increasing in total phenolic content, IC_{50} value in DPPH radical scavenging decreased and antioxidant activity was increased.

3.3. Antimicrobial activity

Antioxidants Recent studies on the essential oils of many *Lamiaceae* family show that these plants have a broad range of biological activities, notably their antimicrobial potency, and this activity is generally correlated to the chemical composition of the oil (Baratta et al., 1998). The antimicrobial activity of *Zhumeria majdae* essential oils against microorganisms which are considered in this study was assessed by evaluating the presence of inhibition zone (IZ) and minimum inhibitory concentration (MIC) values.

The diameters of inhibition zones (IZ) and minimum inhibitory concentration (MIC) values of the essential oil and standard antibiotics used are shown in Table 2. The results showed that the oil exhibited high antimicrobial activity against Gram positive bacteria (except for *E. faecalis*) and low to moderate activity against the Gram negative organisms.

The data showed that *B. cereus* and *B. thuringiensis* were the most sensitive microorganisms to the oil with the inhibition zones of 33 and 30 mm and MIC values of 0.93 mg/ml, respectively. *C. jejuni* and *P. fluorescens* the most resistant organisms to the essential oil. The antimicrobial activity of *Zhumeria majdae* could be attributed to high amount of major components as Linalool, Camphor and other components in essential oil. Linalool and Camphor, which is the main component of *Zhumeria majdae* essential oil and extract, have been considered as biocidal, resulting in bacteria membrane perturbations that lead to leakage of intracellular ATP and potassium ions and ultimately cell death (Juven et al., 1994) . However, it was also considered that, other components as well as a possible interaction between the substances could also affect the antimicrobial activities. In fact, the antimicrobial activity of essential oils may well be due to the presence of synergy, antagonism or additive effects of the other components of the oil which possess various potencies of activity (Didry et al., 1993).

Table 2. Antimicrobial activity of the essential oil from *Z. majdae*

Microorganism	Inhibition Zone (IZ)	Minimum inhibitory concentration (MIC)
Bacillus cereus	33.14 ±0.09	0.93
Bacillus thuringiensis	30.29 ± 0.01	0.93
Enterococcus faecalis	17.05 ± 0.04	7.5
Staphylococcus epidermidis	24.84 ± 0.04	5
Salmonella typhimurium	10.22 ± 0.07	10
Campylobacter jejuni	7.84 ± 0.05	15
Klebsiella pneumoniae	11.03 ± 0.01	10
Pseudomonas fluorescens	6.42 ± 0.02	15

*Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

4. Discussion

Since pre-historic times, man has gone in different ways to search for cures and relief from various diseases by using numerous plants, plant products and plant-derived products .Recently, there is a scientific interest and certain popularity with regard to screening essential oils and extracts from plants used medicinally all over the world. Historically, many plants 'essential oils and crude extracts have been used as topical antiseptics, or have been reported to have antimicrobial properties (Akin et al., 2012; Bachir and Benali, 2012; Ćavar et al., 2012; Diao et al., 2013; Diao et al., 2014; Jordán et al., 2013). It is very important now a days to investigate scientifically those plants ,which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Mitscher et al., 1987).

Also, the resurgence of interest in natural control of phytopathogens and increasing consumer demand for effective, safe, and natural products means that quantitative data on plant oils and extracts are required. Various publications have documented the anti-fungal and antibacterial activity of essential oils and plant extracts including rosemary, peppermint, bay, basil, tea tree, celery seed and fennel (Morris et al., 1979).

The hydro distillation of the aerial parts of *Zhumeria majdae* oils with the major components of the oil having oxygenated mono and sesquiterpenes, and their respective hydrocarbons. Recently several researchers have reported that mono and sesquiterpene hydrocarbons and their oxygenated derivatives are the major components of essential oils of plant origin, which have enormous potential to strongly inhibit microbial pathogens (Aligiannis et al., 2001; Mahboubi et al., 2012; Mahboubi & Feizabadi, 2009; Mohaddese & Nastaran, 2009; Omidpanah et al., 2015; Sharififar et al., 2007). In general, the active antimicrobial compounds of essential oils are terpenes, flavonoids which are phenolic in nature, and it would seem reasonable that their antimicrobial or antibacterial mode of action might be related to that of other compounds (Hossain et al., 2012)

The essential oils of *Zhumeria majdae* show antibacterial effect against all the bacterial tested. Some earlier papers on the analysis and antibacterial properties of the essential oils of some species of various genera have shown that they have a varying degree of growth inhibition effects against some Staphylococcus, Bacillus species due to their different chemical compositions (Arman et al., 2009; Mohaddese & Nastaran, 2009; Sharififar et al., 2007). The results of this studyshow that, Linalool and Camphor are the main components in *Z. majdae* essential oils. This finding was same to previous study on essential oil in this plant grown in Iran (Sharififar et al., 2007). Also our study suggested that *Z. majdae* have high phenolic content and antioxidant activity, these results suggest that the major part of the antioxidant activity in *Z. majdae* results from the phenolic compounds.

Antimicrobial properties of the essential oil of *Z. majdae* show that the plant has potential for use in aromatherapy, pharmacy and also in pathogenic systems to prevent the growth of microbes. Thus, *Z. majdae* could become an alternative to synthetic bactericides for use in agro industries and also to screen and develop such novel types of selective and natural bactericides in the treatment of many microbial phytopathogens causing severe destruction to crops, vegetables and ornamental plants.

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