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# CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF *ZHUMERIA MAJDAE* RESH. F.& WENDELBO

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**Abstract**
*Zhumeria majdae* Resh. f.& Wendelbo. (Lamiaceae) is a medicinal plant which has long been used in traditional medicine as antispasmodic, antimicrobial, carminative especially in infants and for dysmenorrheal. The therapeutic benefits of medicinal plants are often attributed to their antioxidant properties. The present study was conducted to evaluate in vitro antibacterial and antioxidant properties of essential oil and various extracts of *Zhumeria majdae* Resh. f & Wendelbo. The GC and GC–MS analysis of the essential oil were resulted in the determination of 26 components representing 97.2% of the oil. Linalool (53.28%) and camphor (26.15%) were the main components. The essential oil has exhibited antibacterial activity against all tested bacteria. Antioxidant activities of the samples were determined DPPH and β-carotene/linoleic acid methods. The essential oil and methanol extract reduced the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) with an IC50 of $20.5 \pm 1.6$ and $26.1 \pm 1.5$ respectively. In the latter method, the methanol extract and essential oil have shown the most activity with 25.8 and 19.2 mm mean zone of color retention respectively. The amount of the total phenolics was highest in methanol extract ($50.1 \pm 2.3$μg/mg). A positive correlation was observed between the antioxidant activity potential and total phenolic level of the extracts. Results here show that the essential oil and methanol extract of *Z. majdae* possess antioxidant and antibacterial activity, and could be used as natural preservative in food and /or pharmaceutical industries. The other extracts have exhibited no activity.

**Keywords**:
Antioxidant activity, Antibacterial activity, β-carotene/linoleic acid, DPPH.

## Introduction

Oxidative damages in the human body play an important causative role in disease initiation and progression (1). Antioxidants can protect the human body from free radicals effects and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods (2,3). Therefore, the development and utilization of more effective antioxidants are desired (4-6) and antioxidant supplements may be used to help the human body to reduce oxidative damage (7-9). Plants belonging to the Lamiaceae due to high percent of essential oil, have been used more than other plants as flavoring agents or for medicinal uses.

The volatile compounds have great application and demand in food, perfumery,

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cosmetics, pharmaceutical and winery industries and using of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum, both for the growing interest of consumers in ingredients from natural sources and also because of increasing concern about potentially harmful synthetic additives (10). The monotypic Iranian *Zhumeria majdae* Resh. f. & Wendelbo (*Z. majdae*), known locally by the name of Mohrekhosh, has been described as the first member of a new genus (*Zhumeria*) (11). It has a limited geographical range in Bandar-abbas in Hormozgan province in southeastern of Iran (12).

The leaves have been used for many years as a curative for stomachaches, as an antiseptic, carminative especially in infants and for treatment of painful menstruation (13).

This plant belongs to the Lamiaceae family and has a strong and pleasant odor. Despite its wide uses in traditional medicine, there are few reports of this plant in the literature. The extract of aerial parts of the plant has shown anti-inflammatory and antinociceptive effects in mice and rats (14). The ethanol extract of *Zhumeria majdae* has shown potent antileishmanial and antiplasmodial activity in vitro (15). Antibacterial activity of the essential oil of *Z.majdae* has been previously reported only against two bacterial strains of *S. aureus* and *E .coli* (16). The aim of the present work was to study in vitro antioxidant and antibacterial activities of the essential oils and various extracts of *Z. majdae* and to determine the chemical composition of its essential oil by GC/MS. Additionally total phenolic and flavonoid contents of the various extracts of the plant have been determined. Therefore, the essential oil and various extracts were screened for their possible antioxidant activities by two complementary test systems, namely DPPH free radical-scavenging and beta carotene/ linoleic acid.

## Materials and methods

*Plant material*

*Zhumeria majdae* Resh. f.& Wendelbo. (Lamiaceae) tops were collected from plants wild growing in Bandar-abbas (Hormozgan province) at the full flowering stage (June & July 2006). The taxonomic identification of plant materials was confirmed by Dr. Mozaffarian in the department of Botany of the Research Institute of Forests and Rangelands (TARI). Voucher specimens were deposited in the Herbarium center of Kerman Faculty of Pharmacy (KF1387), Kerman, Iran.

*Chemicals used*

All the chemicals were of analytical grades and were obtained from various sources. Diphenylpicrylhydrazil (DPPH), beta carotene, linoleic acid and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (Taufkirchen, Germany). Analytical thin-layer chromatography was carried out on silica gel $G_{60}$ (35-70 µm, Merck) and bacterial strains were prepared from the department of Pharmaceutics, faculty of pharmacy, Kerman university of medical science.

*Phytochemical screening*

The plant of *Zhumeria majdae* was subjected to phytochemical studies for searching of the flavonoids, alkaloids, tannins and saponins (17).

*Isolation of the essential oil*

The air-dried and ground herbal parts of the plant collected were submitted for 4 h to water-distillation using Cleavenger apparatus. The obtained essential oil was dried over anhydrous sodium sulphate, then stored at +4 °C until tested and analyzed.

*Preparation of the various extracts*

Top flowerings of the plant were dried in the shade, ground in a grinder with a 2 mm in diameter mesh, and about 500g of dry powder was extracted using percolation method with petroleum ether, chloroform, methanol and water respectively for 48h. Solvent removal carried out under vacuum to drying.

*Total phenolics content*

Total phenolics content of the extracts was determined using Folin–Ciocalteau method with little change (18). Briefly, 0.5 ml diluted extract was shaken for 1 min with 100 µl of Folin–Ciocalteau reagent and 6 ml of distilled water. After the mixture was shaken, 2 ml of 15% $Na_2CO_3$ was added and the mixture was shaken once again for 0.5 min. Finally, the solution was brought up to 10 ml by adding distilled water. After 1.5 h, the absorbance at 750 nm was evaluated using a spectrophotometer. The results were expressed as Gallic acid equivalents.

*Assay for total flavonoids*

Total flavonoid content of methanol extracts of *Z. majdae* was based on the method described by Moreno (19) with slight modifications. An aliquot of 1 ml of the solution containing 1 mg extracts in methanol was added to test tubes containing 0.1 ml of 10% aluminium nitrate, 0.1 ml of 1 M potassium acetate and 3.8 ml of methanol. After 40 min at room temperature, the absorbance was read spectrophotometrically at 415 nm. Quercetin was used as a standard (20).

*Gas chromatography/mass spectrometry analysis*

*Gas chromatography analysis*

The essential oil was analyzed using a Shimadzu QP 5000 gas chromatograph equipped with a FID detector and HP-5 MS_capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Injector and detector temperatures were set at 220 and 290 °C, respectively. Oven temperature was kept at 50 °C for 3 min, then gradually raised to 160 °C at 3 °C/min, held for 10 min and finally raised to 240 °C at 3 °C/min. Helium was the carrier gas, at a flow rate of 1 ml/min. Diluted sample (1/100 in acetone, v/v) of 1.0 µl was injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

*Gas chromatography/mass spectrometry analysis*

GC–MS analysis of the essential oil was performed under the same conditions with GC (column, oven temperature, flow rate of the carrier gas) using a Shimadzu QP 5000 gas chromatograph equipped with a Shimadzu QP 5050 mass selective detector in the electron impact mode (70 eV). Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively.

The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley 2001 library data of the GC–MS system and literature data (21).

Alkanes were used as reference points in the calculation of relative retention indices (RRI).GC and GC/MS analysis results are shown in Table 1.

*Antibacterial activity*

*Microbial strains*

The essential oil and various extracts were individually tested against a panel of microorganisms including *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Staphylococcus epidermidis* ATCC14990, *Bacillus subtilis* ATCC 6051 and *Pseudomonas aeruginosa* ATCC 6027. Bacterial strains were cultured overnight in 37°C in Mueller Hinton agar (MHA).

*Assay for inhibition of bacterial growth*
The antibacterial activities of the essential oil and various extracts from the plant were determined by the hole-plate diffusion method (22). The tested bacterial suspension was homogeneously seeded onto Petri dishes containing 15 ml of the MHA medium. Holes were aseptically bored into agar with a hollow punch and 25 µl aliquots of the samples (200mg/ml) which were dissolved in dimethylsulfoxide (DMSO) were placed into wells with a sterile pipette. The plate was kept for 1h at room temperature for the diffusion of the samples into the agar takes place. Subsequently, the plate was incubated at 37ºC for 18h. Gentamicin and DMSO were used as positive and negative control respectively. The microorganism control consisted of a seeded Petri dish with no plant materials, solvent or gentamicin. Results were recorded as the mean of triplicate experiments. Bacterial growth inhibition was determined as the diameter of the inhibition zone around the holes. The inhibition diameter was the average of three measurements per hole.

*Determination of Minimum Inhibitory Concentration (MIC)*
The MICs were determined by the agar dilution method. Two- fold serial dilutions of the extract (50mg/ml in DMSO) were prepared in MHA.
Solvent, antibiotic and microorganism controls were also analyzed.
Bacterial inoculums (2 µl) were seeded on to the agar. The plates were incubated at 37ºC for 18h. MIC was considered the lowest concentration of the sample that prevents visible growth of microorganism (22).

*Antioxidant activity*
*DPPH assay*
Hydrogen atom or electron-donation ability of the corresponding oil and extracts were measured from the bleaching of the purple-colored methanol solution of DPPH. This spectrophotometric assay uses stable 2, 2′-diphenylpicrylhydrazyl (DPPH) radical as a reagent (23). Fifty µl of various concentrations of the samples in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition of DPPH free radical in percent (I%) was calculated in following way:
$I\% = (A_{blank} - A_{sample}/A_{blank}) x100$

Where $A_{blank}$ is the absorbance of the control reaction (containing all reagents except the test compound), and $A_{sample}$ is the absorbance of the test compound. Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate.

*DPPH assay on TLC*
This procedure was applied for the essential oil and extracts. Five µl of a 1:10 dilution of the extracts in methanol were applied to the TLC plate and a suitable solvents mixture was used as developer.

Table 1: Amount of total phenolics and flavonoid content of various extracts of *Z. majdae*

| Sample | Phenolic content (µg GAs/mg extract)[b] | Flavonoid content (µg QEs/mg extract)[c] |
|---|---|---|
| Petroleum ether extract | - | - |
| Chloroform extract | 3.4 ± 0.2 | - |
| Methanol extract | 50.1 ± 2.3 | 48.4 ± 3.1 |
| Aqueous extract | 27.4 ± 0.9 | 13.5 ± 0.7 |

The plate was sprayed with a 0.2% DPPH reagent in methanol and left at room temperature for 30 min. As explained above, yellow spots formed from bleaching of the purple color of DPPH reagent, were evaluated as positive antioxidant activity (23).

*Beta carotene bleaching test*

The rationale behind this method is that β-carotene is a yellow antioxidant. On exposure to light and oxygen, it becomes colorless. However, in the presence of another antioxidant not sensitive to light or oxygen, it will retain its yellow color for a longer period.

*Preparing the media and test solutions*

Technical agar No. 3 (6 g) was weighed and dissolved in 500 ml of distilled water to produce a 1.2% technical agar solution by boiling. It was then poured into 100 ml amber bottles and sterilized in an autoclave at 121°C for 15 min. It was then stored in a cool dry place away from light until time of use. 40 mg of β-carotene (Sigma) was weighed and dissolved in 20ml of acetone to produce a β-carotene solution of concentration 2 mg/ml. 40 mg of linoleic acid (Sigma) was weighed in a beaker and dissolved in 20 ml of ethanol to produce a linoleic acid solution of concentration 2 mg/ml.

*Procedure*

Linoleic acid solution (10 ml) and β-carotene solution (10 ml) were added to the molten agar (10 ml). This was then shaken to achieve uniform distribution of the added components. An orange color was produced. The agar was then poured into Petri dishes (25 ml per dish). Petri dishes were covered with black plastic to exclude light and left standing to allow the agar to set. Holes (4 mm diameter) were then punched into the agar using a borer. Pure oil (25 μl) was transferred into the holes and Petri dishes were then incubated at 45°C for 4 h. A zone of color retention around the hole after incubation indicated essential oils with antioxidant properties. The zone diameter was measured using vernier calipers. Alcohol was used as a negative control and ascorbic acid (10 mg/ml) was used as a positive control (24).

## Results

*Phytochemical screening*

Phytochemical screening of the plant shows the presence of flavonoids and tannins in the plant.

*Total phenolics and flavonoid content*

*Yield*

It is considered that the phenolic compounds contribute to overall antioxidant activities of *Z. majdae* extracts. The extraction yield of phenolics content varied from 3.4 to 50.1 μg GAs/mg extract depending on the extraction solvent with the following order: methanol > aqueous > chloroform extracts. Total flavonoid content has been found 48.4 μg/mg of extract for the methanol extract (Table 1).

*Chemical composition of the essential oil*

Air-dried herbal parts of *Z.majdae* were subjected to hydro distillation using a Clevenger apparatus and the pale yellow-colored essential oil was obtained (yield 8.3% v/w).The results obtained by GC–MS analysis of the essential oil of the plant are presented in Table 2.

Twenty-six compounds were identified, representing 97.74% of the total oil. The oil profile exhibits that linalool was the main compound (53.28%); additionally other major compounds were camphor (26.15%) and D- limonene (4.17%).

Table 2: Chemical composition of Zhumeria majdae essential oil (diluted 1/100 in acetone v/v)[1]

| Peak No. | components | KI[2] | %composition |
|---|---|---|---|
| 1 | Alpha- pinene | 939 | 0.87 |
| 2 | camphene | 953 | 2.16 |
| 3 | beta- pinene | 982 | 0.05 |
| 4 | 3- octanone | 986 | 0.94 |
| 5 | beta-myrcene | 991 | 0.48 |
| 6 | un known | 999 | - |
| 7 | octanal | 1001 | 0.14 |
| 8 | alpha-terpinene | 1019 | 0.08 |
| 9 | para cymene | 1026 | 0.37 |
| 10 | D-limonene | 1031 | 4.17 |
| 11 | beta-phelandrene | 1033 | 0.06 |
| 12 | trans-ocimene | 1050 | 0.17 |
| 13 | gamma-terpinene | 1062 | 0.58 |
| 14 | cis-linalool | 1074 | 0.53 |
| 15 | L-linalool | 1099 | 53.28 |
| 16 | camphor | 1143 | 26.15 |
| 17 | borneol | 1165 | 1.32 |
| 18 | 4- terpineol | 1177 | 0.4 |
| 19 | Alpha- terpineol | 1189 | 0.7 |
| 20 | unknown | 1204 | - |
| 21 | cis geraniol | 1228 | 0.25 |
| 22 | citral | 1242 | 0.53 |
| 23 | trans geraniol | 1255 | 2.4 |
| 24 | geranial | 1270 | 1.01 |
| 25 | unknown | 1276 | - |
| 26 | thymol | 1290 | 0.07 |
| 27 | unknown | 1296 | - |
| 28 | unknown | 1394 | - |
| 29 | beta caryophyllene | 1418 | 0.27 |
| 30 | Caryophyllene oxide | 1581 | 0.2 |
| 31 | beta-eudesmol | 1649 | 0.07 |
| Total | | | 97.2 |

[1]Relative percentages of the compounds were obtained electronically from FID area percent data
[2]Kovats index on non-polar DB-5 ms column in reference to *n*-alkanes

It is quite possible that most of the pharmacologic effects of this plant for example antispasmodic, carminative and sedative would be pertained to its high contents of linalool and camphor. Linalool has sedative, anti nociceptive and anti inflammatory activities (25-27) and camphor has been reported to be analgesic and as an antimicrobial agent (28-30).

*Antibacterial activity*

According to the results given in Table 3, the essential oil of *Z .majdae* exhibited notable antibacterial activity against all the bacterial species tested in the concentration of 0.39 mg/ml.

The petroleum ether extract was also found to be effective against some strains. The antibacterial spectrum of this extract is similar to the essential oil, but with lesser activity, probably due to the presence of similar compounds in these two fractions. Based on the results of chemical composition of the essential oil, it can be concluded that the antibacterial nature of the essential oil studied is apparently attributed to its high linalool and camphor contents (53.28 % and 26.15% respectively). The antimicrobial activity of linalool and camphor has been reported previously (30,31). The methanol extract has shown most activity against *P.*

*aeruginosa.* This activity may be pertained to the presence of flavonoids (25), and more polar thermo-labile and/or thermo-stable phenolics (32).

*Antioxidant activity*

*DPPH assay and TLC*

Free radical scavenging activities of the essential oil and various extracts are given in Table 4. In essence, the antioxidants react with the stable free radical i.e. 1, 1-diphenyl-2-picrylhydrazyl (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. The degree of discoloration indicates the free radical scavenging potentials of the sample (33).

The essential oil was able to reduce the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to the yellow-colored diphenylpicrylhydrazil with an $IC_{50}$ of 20.5 ± 1.6, exhibiting almost similar activity to the synthetic antioxidant agent BHT (17.9 ± 1.7). Among the various extracts of the plant, methanol extract showed the most activity (26.1± 1.5). TLC analysis showed the essential oil and methanol extract exhibit the most persistent color respectively. The analysis of the essential oil shows that most of the composition of this oil includes hydrocarbon and oxygenated monoterpenes (95.51%).

*Beta carotene- linoleic acid method*

In β-carotene/linoleic acid model system, β-carotene undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. The linoleic acid free radical formed by the abstraction of a hydrogen atom from one of its diallylic methylene group attacks the highly unsaturated β-carotene molecules. As a result, β-carotene is oxidized and breaks down in parts; subsequently the system loses its chromophore and characteristic orange color, which is monitored spectrophotometrically. In this test, the methanol extract and essential oil from *Z. majdae* exhibited significant antioxidant properties. They were able to inhibit the discoloration of beta carotene with 25.8 and 19.2 mm mean zone of color retention respectively (Table 5). The methanol extract has been effective in both antioxidant methods. By considering the results of phytochemical screening and total phenolics and flavonoids content, the activity of the methanol extract would be mostly attributed to these compounds. The key role of phenolic compounds as antioxidant and scavengers of free radicals is emphasized in several reports (34-36).

Table 3: Minimum Inhibitory concentrations (mg/ml) of essential oil and various extracts from *Z. majdae* against tested microorganisms

| Species or reference compound | *S. aureus* | *S. epidermidis* | *B. subtilis* | *E. coli* | *K. pneumonia* | *P. aeruginosa* |
|---|---|---|---|---|---|---|
| Essential oil | 0.02 | 0.195 | 0.39 | 0.39 | 0.39 | 0.195 |
| Petroleum ether ex. | 12.5 | 6.25 | NA | 25 | 12.5 | 50 |
| Chloroform ex. | 12.5 | 6.25 | 12.5 | 25 | 25 | 50 |
| Methanol ex. | 12.5 | NA | NA | 12.5 | 25 | 6.25 |
| Aqueous ex. | NA | NA | NA | 25 | 12 | NA |
| Gentamicin (μg/ml) | 1.5 | 2 | 1 | 0.25 | 0.5 | 0.5 |

## Discussion

In order to prolong the storage stability of foods and reduce the damage to human body, synthetic antioxidants are used for industrial processing. But according to toxicologists and nutritionists, the side effects of some synthetic antioxidants such as BHT have already been documented.

Therefore, governmental authorities and consumers are concerned about the safety of their food and the potential effects of synthetic additives on health (10).

We have reported previously the presence of some interesting antioxidants from the endemic plants of Iran especially from Lamiaceae family (37,38). Results presented here are the first works on the antioxidant activity of Zhumeria *majdae*. The essential oil and methanol extract of this plant have exerted strong antioxidant activities almost equal to BHT. The aerial parts of the plant were never investigated.

Phytochemically, only in the root of the plant, labdane diterpenes have been detected (39) Labdane diterpenes have been detected as a potential radical scavenger by Tapia (40).

The essential oil and methanol extract from this plant also exhibited antibacterial activity. Linalool (53.28%) and Camphor (26.155%) could be assumed responsible for antibacterial effects of the essential oil. An antimicrobial activity of linalool and camphor has been reported previously (41, 42). It seems to be a general trend that the essential oils which contain monoterpene hydrocarbons, oxygenated monoterpenes and/or sesquiterpenes have greater antioxidative properties and monoterpenes found in this essential oil may act as radical scavenging agents (23).

As expected, the amount of total phenolics was highest in methanol extract (50.1 ± 2.3 μg/mg). It is extremely important to point out that there is a positive correlation between the antioxidant activity potential and the amount of phenolic compounds of the extracts. Thus methanol extract which is rich of phenolics and some flavonoids could be used safely in food and pharmaceutical industries. In conclusion, our study can be considered as the first report on the in vitro antibacterial and antioxidant activity of *Z. majdae*. Owing to the strong antibacterial and excellent protective features of the essential oil exhibited in antioxidant activity tests, and by considering the high percent of essential oil in this plant (8.3%), it is evident that the biologic activity of the plant depends mostly to its essential oil and the herbal parts of *Z.majdae* can be freely used in the food and pharmaceutical industry as preservative from a natural source.

Table 4: DPPH assay of the essential oil and various extracts from *Z.majdae*

| Sample | $IC_{50}$ |
|---|---|
| Essential oil | 20.5 ± 1.6 |
| Petroleum ether extract | 40.1 ± 1.6 |
| Chloroform extract | 45.8 ± 2.2 |
| Methanol extract | 26.1± 1.5 |
| Aqueous extract | 53.6 ± 3.1 |
| BHT | 17.9 ± 1.9 |

Table 5: Antioxidant activity of the essential oil and various extracts from *Z. majdae* given in terms of mean zone of inhibition (mm)

| Sample | Mean zone of inhibition(mm) |
|---|---|
| Essential oil | 25.8 ± 0.8 |
| Petroleum ether extract | 13.1 ± 1.6 |
| Chloroform extract | 4.2 ± 0.2 |
| Methanol extract | 19.2± 1.5 |
| Aqueous extract | 6.7 ± 1.1 |
| BHT | 29.4 ± 1.2 |

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