Chemical composition and antimicrobial activity of *Micromeria hedgei* Rech. f. oil from Iran

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Abstract

*Micromeria hedgei* belongs to the Lamiaceae family is a rare endemic and endangered species that has been used in traditional medicine in Iran. In this regard essential oil composition and antimicrobial activity of wild and cultivated *M. hedgei* was reported for the first time. Essential oils isolated via hydro distillation from the aerial parts of *M. hedgei* were analyzed by a combination of capillary GC and GC–MS. The major constituents were geranial (18.04 and 22.68%), neral (13.81 and 15.99%), geraniol (13.15 and 10.74%), nerol (7.69 and 6.02%), E-caryophyllene (6.52–3.80%), carvacrol (6.20 and 5.27%), geranyl acetate (5.79 and 3.06 %), caryophyllene oxide (4.73 and 3.88 %), thymol (3.13 and 3.63%), and α-humulene (3.27 and 3.27%) in wild and cultivated *M. hedgei*. Antimicrobial activity of essential oils was investigated by disc diffusion method. Essential oil showed good antimicrobial activity against five medically important pathogens compared with standard antibiotics.

Keywords: *Micromeria hedgei*, essential oil, antimicrobial, geranial, neral
Experimental

3.1. Plant material

Aerial parts of *M. hedgei* were collected in April 2015 in full-flowering stage in natural habitat of Bokhoon area Hormozgan province (Near Persian Gulf) Southern Iran and cultivated plants in Medicinal and Aromatic Plants Experimental Garden (MAPEG) of the Estahban branch, Islamic Azad University in Fars province in southwest Iran (Fig S1). The plants were identified and authenticated (voucher no.116) at the herbarium of medicinal and aromatic plants of (IAU), Estahban branch, Fars, Iran. Climatic conditions of *M. hedgei* habitats were determined using the nearest meteorology station (Table S1). The harvested plants in different habitats were dried at room temperature (25°C) for 2 weeks. Then, air-dried plants ground and powdered with mixer for essential oil extraction.

3.2. Essential oil extraction

Dried aerial parts were ground into powder (mesh< 35), and 100 g of the powdered tissue was distilled with 1 L of water for 3 h using a Clevenger-type apparatus according to the method recommended in the British Pharmacopoeia (*British Pharmacopoeia*, 1988). The oils were dried over anhydrous sodium sulfate, weighed, and stored in dark glass vials at 4°C prior to analysis and antimicrobial tests.

3.3. Identification of the Oil Components

The essential oil composition was determined by GC and GC-MS analysis. The analysis was performed using a gas chromatograph (Agilent Technologies 7890 GC) equipped with a FID detector, using HP-5MS 5% capillary column (30 m × 0.25 mm, 0.25 μm film thicknesses). The carrier gas was Helium at a flow of 1 ml/min. Initial column temperature was 60°C and was programmed to increase at 3°C/min to 280°C. The injector and detector temperatures were set at 280 °C. The split ratio was 20:1. Oil samples (0.2 μl) were injected manually. The percentage compositions were obtained from electronic integration of peak areas without the use of correction factors.

The GC-MS analysis was done on the Agilent Technologies 5975 Mass system. The EI-MS operating parameters were as follows: ionization voltage, 70 eV; ion source temperature, 200 °C. The retention indices for all the components were determined according to the Van Den Doll method using n-alkanes as standard (*Van Den Dool and Kratz, 1963*). The compounds were identified by comparison.
of retention indices (RRI- HP-5) with those reported in the literature and by comparison of their mass spectra with the Willey and mass finder 3 libraries or with the published mass spectra (Adams, 2001).

3.4. Microorganisms

Standard strains of *Candida albicans* (ATCC 10231), the Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 1435), *Bacillus cereus* (ATCC 1247) and the Gram-negative bacterium *Escherichia coli* (ATCC 25922) were all obtained from the Iranian Research Organization for Science and Technology.

3.5. Determination of antimicrobial activity by the disk diffusion method

In vitro antimicrobial activities of the essential oils of *M. hedgei* were evaluated by the disk diffusion method, with determination of inhibition zones (IZ), using Mueller-Hinton agar for bacteria (MHA) and Sabouraud dextrose agar (SDA) for fungi (Baron and Finegold, 1990). Fungal or bacterial suspension were seeded into Petri dishes (9 cm) containing 20 mL of growth medium using a sterile cotton swab. The sterile paper discs (6 mm in diameter) were individually impregnated with 10 μL of the oil and then placed on the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37°C for 24 h and at 24°C for 48 h for the *C. albicans* strain. All studies were performed in triplicate. Blank disks containing 10 μL DMSO were used as negative controls. Nystatin (30 μg/disk), Tetracycline (30 μg/disk), Ketoconazole (20 μg/disk), and Gentamicin (30 μg/disk) were used as positive reference standards to determine the sensitivity of the microorganisms.

A broth micro-dilution method was used to determine the minimum inhibitory concentration (MIC) according to the National Committee for Clinical Laboratory Standards (NCCLS, 2001). A serial double dilution of the oil was prepared in a 96-well micro-titer plate over the range of 0.02–50.00 μL/mL. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. All determinations were performed in triplicate.
### Table S1. Geographical and environmental conditions of *M. hedgei* growing wild and cultivated in Iran

| Region | Province | Altitude (m) | Latitude (UTM) | Longitude (UTM) | Temp.°C | Rainfall | E.C. | Ph | R.H.% | S.T. | 
|--------|----------|--------------|----------------|----------------|---------|----------|------|----|-------|------| 
| Bokhoo | Hormozgan| 1600         | 27°56’41"N     | 56°16’34”E     | 23.21   | 212      | 4.20 | 7.79| 76.7  | Loam | 
| Estahban| Fars     | 1760         | 29°71’26"N     | 54°13’57”E     | 20.50   | 300      | 2.80 | 7.22| 35.9  | Sandy loam | 

*°*: Average temperature  **°**: Average annual Rainfall  **°**: Electrical conductivity  **°**: Relative humidity  **°**: Soil texture

Meteorological information was obtained from the nearest meteorology station within the study area and the surrounding zone; each value in the mean of 10 years data. Physical and chemical Soil characteristics are based on average of three samples taken from each region.
Table S2. Essential oil constituents of *M. hedgei* growing wild and cultivated in Iran

| No | Compound              | RI* | % Bokhoon (Natural habitat) | % Estabhan (Cultivated plants) |
|----|-----------------------|-----|-----------------------------|--------------------------------|
| 1  | α-Pinene              | 932 | 0.49                        | 0.17                           |
| 2  | Sabinene              | 972 | 1.10                        | 0.47                           |
| 3  | β-Pinene              | 976 | 2.90                        | 1.02                           |
| 4  | Myrcene               | 990 | 0.46                        | 0.37                           |
| 5  | δ-3-Carene            | 1010| 0.10                        | 0.05                           |
| 6  | α-Terpinene           | 1016| 0.06                        | 0                              |
| 7  | p-Cymene              | 1023| 0.36                        | 0.07                           |
| 8  | Limonene              | 1027| 1.81                        | 1.15                           |
| 9  | 1,8-Cineole           | 1030| 0.07                        | 0.05                           |
| 10 | (Z)-β-Ocimene         | 1036| 0.07                        | 0.07                           |
| 11 | Benzene acetaldehyde  | 1042| 0.08                        | 0                              |
| 12 | (E)-β-Ocimene         | 1046| 0.34                        | 0.76                           |
| 13 | γ-Terpinene           | 1057| 0.36                        | 0.08                           |
| 14 | cis-Sabinene hydrate  | 1066| 0.08                        | 0.13                           |
| 15 | trans-Linalene oxide  | 1072| 0.11                        | 0.04                           |
| 16 | cis-Linalene oxide    | 1089| 0.10                        | 0.09                           |
| 17 | Linalool              | 1099| 2.23                        | 2.04                           |
| 18 | n-Nonanal             | 1104| 0.26                        | 0.15                           |
| 19 | trans-Pinocarveol     | 1138| 0.60                        | 0.19                           |
| 20 | Menthol               | 1154| 0.51                        | 0.36                           |
| 21 | Pinocarvone           | 1163| 0.21                        | 0.25                           |
| 22 | Rosefuran epoxide     | 1175| 0.19                        | 0                              |
| 23 | Terpinene-4-ol        | 1177| 0.17                        | 0.33                           |
| 24 | α-Terpinol            | 1191| 0.46                        | 0.42                           |
| 25 | Myrtenol              | 1197| 0.42                        | 0.24                           |
| 26 | trans-Cardol          | 1219| 0.10                        | 0.11                           |
| 27 | Nerol                 | 1228| 7.69                        | 6.02                           |
| 28 | Neral                 | 1241| 13.81                       | 15.99                          |
| 29 | Carvone               | 1244| 0.27                        | 0.13                           |
| 30 | Geraniol              | 1255| 13.15                       | 10.74                          |
| 31 | Geranial              | 1270| 18.04                       | 22.68                          |
| 32 | Thymol                | 1292| 3.13                        | 3.63                           |
| 33 | Carvacrol             | 1300| 6.20                        | 5.27                           |
| 34 | α-Cubebene            | 1349| 0.13                        | 0.32                           |
| 35 | Eugenol               | 1358| 0.23                        | 0.17                           |
| 36 | α-Copaene             | 1376| 2.20                        | 2.29                           |
| 37 | Geranyl acetate       | 1385| 5.79                        | 3.06                           |
| 38 | β-Cubebene            | 1390| 0.28                        | 0.56                           |
| 39 | (E)-Caryophyllene     | 1419| 3.80                        | 6.52                           |
| 40 | α-Humulene            | 1453| 3.27                        | 6.39                           |
| 41 | Germacrene D          | 1480| 0.32                        | 0.12                           |
| 42 | (E)-β-Ionone          | 1486| 0.10                        | 0.11                           |
| 43 | (E,E)-α-Farnesene     | 1509| 0.09                        | 0.14                           |
| 44 | δ-Cadinene            | 1523| 0.93                        | 1.56                           |
| 45 | α-Calacorene          | 1543| 0.13                        | 0.14                           |
| 46 | Spathulenol           | 1577| 0.18                        | 0.09                           |
| 47 | Caryophyllene oxide   | 1582| 4.73                        | 3.88                           |
| 48 | n-Hexadecane          | 1599| 0.33                        | 0.27                           |
| 49 | Humulene epoxide II   | 1608| 1.35                        | 1.02                           |

Monoterpane hydrocarbons 8.05  4.21
Oxygenated monoterpenes 73.9  72.09
Sesquiterpene hydrocarbons 11.15  18.04
Oxygenated Sesquiterpene 6.26  4.99
Others 0.43  0.38
Total 99.79  99.71
Essential Oil Yield (% W/W)  
1.12 ±0.13 b  2.23±0.16 a  

1. RI retention indices in elution order from HP-5 column. Data expressed as percentage of total. Essential oil yield was obtained by calculating the average of three experiments ±standard deviation. Essential oil yield was obtained by calculating the average of three experiments ±standard deviation.

Table S3. Essential oil constituents of some *Micromeria* species

| Species         | Main components                                                                 | References                        |
|-----------------|----------------------------------------------------------------------------------|-----------------------------------|
| *M. hedgei*     | geranial (18.04 and 22.68%), neral (13.81 and 15.99%), geraniol (13.15 and 10.74%), nerol (7.69 and 6.02%), E-caryophyllene (6.52–3.80%), carvacrol (6.20 and 5.27%) | Present study                     |
| *M. dichodontha*| β-caryophyllene (42.56%)                                                         | (Baser et al., 1992)              |
| *M. myrtifolia* | pulegone (57.2%, 81.3% and 39.6%)                                               | (Özek et al., 1992).              |
| *M. fruticosa*  | pulegone (32.8%) and piperitenone (25.7%)                                      | (Kirimier, 1992).                 |
| *M. albanica*   | piperitenone oxide (41.8% and 38.7%) and pulegone (15.9% and 13.4%)              | (Marinkovic et al., 2002).        |
| *M. juliana*    | Carvacrol                                                                        | (Phokas et al., 1980).            |
| *M. jana*       | isoegenol (31.5%)                                                               | (Mastelic et al., 2005)           |
| *M. graeca*     | Caryophyllene oxide (17.0%), epi-α-bisabolol (12.8%) and linalool (18.1%) and β-caryophyllene (12.5%) | (Tzakou and Coulaidis, 2001).    |
| *M. sinica*     | Isoeugenol (15.2%), α-pinene (15.0%) and (E)-nerolidol (13.8%)                   | (Hawary et al., 1991).            |
| *M. browni*     | Pulegone (51.7%), menthone (20.9%) and neomenthol (11.9%)                       | (Masoudi et al, 2009).           |
| *M. fruticosa*  | Piperitenone oxide (50.6%) and pulegone (29.2%)                                 | (Tucker et al., 1992).            |
| *M. persica*    | Thymol (33.1% and 28.6%), γ-terpinene (28.7% and 17.5%), limonene (5.0% and 20.7%), 1,8-cineole (14.2% and 0.2%) and p-cymene (7.0% and 17.5%) | (Gulluce et al., 2004).          |
| *M. congesta*   | Piperitenone oxide (40–45%), pulegone (9.7–11.8%) and verbenone (8.3–9.4%)     | (Sefidkon and Kalvandi 2005).     |
| *M. cristata*   | Camphor (9-15%), caryophyllene oxide (4-6%), and trans-verbenol (4-6%)           | (Tabanca et al., 2001).           |
| *M. biflora*    | Neral (25.3–32.2%) and geranial (26.7–41.3%)                                   | (Mallavarapu et al, 1997).        |
| *M. carminea*   | Bornol (26.0%)                                                                  | (Baser et al., 1995).             |
| *M. albanica*   | Piperitenone oxide (44%)                                                        | (Stojanovic et al., 1999).        |
| *M. juliana*    | Verbenol (11.8%), thymol (10.8%) and caryophyllene oxide (10.5%)                | (Stojanovic et al., 2006).        |
| *M. croatica*   | Caryophyllene oxide                                                             | (Kremer et al., 2012).            |
| *M. cilicica*   | Pulegone (64.10–66.55%)                                                         | (Duru et al., 2004)               |
**Table S4. Antimicrobial activity of the essential oil of *M. hedgei***

| Microorganism | Plant condition | Standard antibiotics |
|---------------|-----------------|----------------------|
|               | Natural habitat | Cultivated plants    | Tetracycline (30 μg/disk) | Nystatin (30 μg/disk) | Ketoconazol (20 μg/disk) | Gentamicin (30 μg/disk) |
| *C. albicans* | 17 b 3.12 a     | 21 b 1.56 a          | ---                       | 17                     | 21                     | ---                     |
| *S. aureus*   | 19 a 1.56 b     | 25 a 0.78 b          | 20                       | ---                    | 20                     | ---                     |
| *S. epidermidis* | 19 a 1.56 b | 24 a 0.78 b          | 30                       | ---                    | 18                     | ---                     |
| *E. coli*     | 18 a 1.56 b     | 23 a 0.78 b          | ---                      | ---                    | ---                    | 22                      |
| *B. cereus*   | 19 a 1.56 b     | 25 a 0.78 b          | 20                       | ---                    | ---                    | ---                     |

*a Diameter of inhibition zones (mm) including diameter of sterile disk (6 mm). Essential oil was tested at 10 μL/disk for each tested microorganism. Each value in the table was obtained by calculating the average of three experiments. Values followed by the same letter under the same row, are not significantly different (p > 0.05).*

*b Minimum inhibitory concentration, values as μg/mL. Lower MIC values indicated the highest antimicrobial activity.*
Figure S1. *Micromeria hedgei* growing wild (left) and cultivated (right) in Iran

![Natural habitat](image1) ![Cultivated plant](image2)

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