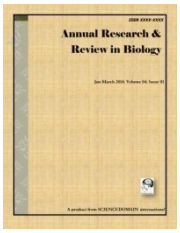

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# Essential Oil Compositions and Antibacterial Properties of Mint (*Mentha longifolia* L.) and Rosemary (*Rosmarinus officinalis*)

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***Authors' contributions***

*Author ZM wrote the protocol, managed the phytochemical analyses and wrote the manuscript. Author AR designed the study and managed the antibacterial analyses of the study. 'Author FB managed the literature searches. All authors read and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

**Aims:** This study was designed to investigate the chemical composition (gas chromatography/mass spectrometry [GC-MS]) and antibacterial (Gram-positive and Gram-negative bacteria) activities of essential oils extracted from aerial parts of two medicinal plants, *Mentha longifolia* L. and *Rosmarinus officinalis,* by MIC and MBC assays.
**Place and Duration of Study:** Institute of Agriculture, Deputy of Research and Executive, University of Zabol, Zabol, Sistan & Blochestan province, Iran, 2013May to2013 October.
**Methodology:** The aerial parts of *Mentha longifolia* L. and *Rosmarinus officinalis* were collected during their flowering stage. Collected plant samples were air dried in shade and under room temperature conditions. 50 g of dried plants were crushed into smaller pieces, and hydrodistilled in a Clevenger–type apparatus for 3h. The extracted compounds were analyzed on a 6890 N Agilent gas chromatograph coupled with a 5975 C Agilent mass-selective detector. For antibacterial activities assay, Minimal Inhibitory Concentration (MIC) tests against *E. coli*, *Pseudomonas aeruginosa*, *salmonella typhi*, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus licheniformis* and *Staphylococcus aureus* were were carried out by using Müller-Hinton broth on a tissue culture test plate. For detection

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of MBC, culturing was done at Nutrient Agar from wells with no bacterial growth and after 24 hr, incubation was done in 37ºC. The plates without bacterial growth were reported as Minimum Bactericidal concentration (MBC).
**Results:** 32 components accounting to 94.27% of the total oil were identified in Mint essential oil and 19 components accounting to 97.5% of the total oil were identified in rosemary essential oil. The major components were alpha-Ocimene, 1, 8-Cineole, Borneol, Geraniol and Camphor in rosemary and Pulegone, Isomenthone, beta- Pinene and 1, 8-Cineole in Mint. The essential oil of mint exhibited higher activity rather than *L. monocytogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus ureuse*. Both oils had the same effect on *E. coli*.
**Conclusion:** The monoterpenes and sesquiterpenes confer the chemical profile of the analyzed essential oil of mint and rosemary samplescausing antibacterial effects.

*Keywords: Mentha longifolia L.; Rosmarinus officinalis; essential oil; GC/MS; antimicrobial properties.*

## 1. INTRODUCTION

The plant extracts and secondary metabolites possess antimicrobial, antifungal, and antiviral activities. Aromatic plants have been known for a very long time and owing to their aromatic and antiseptic properties, they are used as spices and natural food preservatives in perfume industry and aromatherapy and for different medical purposes. Herbs of the Lamiaceae family, like rosemary and mint are well known for their essential oil content to which the antimicrobial activity was attributed [1].

The mints, Mentha species belonging to the family Labiatae (Lamiaceae), are widely distributed in Eurasia, Australia, and South and North Africa [1]. Various species of Mentha have been used as folk remedies for treatment of bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints, due to their anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatharral activities [1,2,3,4,5]. The *Mentha longifolia* (L.) Huds., has been commonly used as a kitchen and medicinal plant for centuries.

Rosemary (*Rosmarinus officinalis* L.) is a very important medicinal and aromatic plant that belongs to the Lamiaceae family and has been cultivated since ancient times. Anthropologists and archaeologists have found evidence that rosemary herbs were used as medicinal, culinary, and cosmetic virtues in the ancient Egypt, Mesopotamia, China, and India [6]. Nowadays, Rosemary is a widely used aromatic and medicinal plant. Rosmarini folium has antibacterial, antioxidant, and antiphlogistic effects. The essential oil enhances the blood-circulation of limbs, has antirheumatic effect, and relieves the neuralgic pains. The active virtues of mint and rosemary depend on abundant volatile oils that contain a wide variety of terpenes and terpenoids [6].

The objectives of this study were to analyze the oil composition and antibacterial (Gram-positive and Gram-negative bacteria) activities of essential oils of *Mentha longifolia* and *Rosmarinus officinalis* growing in Institute of Agricultural, University of Zabol, Zabol, Sistan & Balochestan province, Iran.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The aerial parts of *Mentha longifolia* L. and *Rosmarinus officinalis* were collected and identified during their flowering stage in 2013 May from the Sistan & Blochestan province (Institute of Agriculture, University of Zabol, Iran). Collected plant samples were air dried in shade and under the room temperature conditions, 50 g of dried plants was crushed into smaller pieces, and hydrodistilled in a Clevenger–type apparatus for 3 h. The oils were stored in dark tubes in 4ºC until the analysis time.

### 2.2 Gas Chromatography/mass Spectrometry (GC-MS)

The compounds were analyzed on a 6890 N Agilent gas chromatograph coupled with a 5975 C Agilent mass-selective detector (Agilent Technologies, Avondale, PA, USA). A 7683 Agilent autosampler and 2 µL of the sample were injected in split less mode at 250ºC into a 30 m × 0.25 mm × 0.5 µm DB-5 MS capillary column and were operated by MSD Chemstation Software (Agilent Technologies). The temperature program used for the chromatographic separation was as follows: 50ºC for 2 min, temperature increase at 25ºC/min to 100ºC and hold for 2 min, then temperature increased at 5ºC $min^{-1}$ to 290ºC where it was finally held for 5 min. The carrier gas was helium (99.999%) and was kept at a constant flux of 1.0 ml /min. The mass spectrometer operated in electronic impact ionization mode and the energy of the electrons was kept at 70 eV. The interface was kept at 290ºC. The mass spectrums were obtained at a mass ratio scan range from 100 to 400 m/z to determine the appropriate mass.

### 2.3 Identification of Components

The linear retention indices for all the compounds were determined by co-injection of the sample with a solution containing the homologous series of C8–C22 n-alkanes [7]. The individual constituents were identified by their identical retention indices, referring to the known compounds taken from the literature [8] and by comparison of their mass spectra either with the known compounds or with the Wiley mass spectral database.

### 2.4 Evaluation of Antibacterial Activity

Antibacterial activity of essential oils, isolated from *Mentha longifolia* (L.) Huds., and *Rosmarinus officinalis*, was analyzed using Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests. A collection of seven test organisms, including three Gram positive and four Gram-negative bacterial strains, was used. The groups included one organism of American Type of Culture Collection (ATCC), one organism of Institute of Standards and Industrial Research of Iran (ISIRI) and five organisms of Plat Total Colony Count (PTCC). Table 3 shows the source of the bacterial strains. In this study, we used three Gram- negative bacterium, *E. coli* (PTCC 1533), *Pseudomonas aeruginosa* (ISIRI 275) and *Salmonella typhi* (PTCC 1609), and fourGram- positive bacterium, *Listeria monocytogenes* (PTCC 1163), *Bacillus cereus* (ATCC 11778), *Bacillus licheniformis* (PTCC 1525) and *Staphylococcus aureus* (PTCC 1112). In microbiology, MIC is the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after overnight incubation [9]. MICs were defined as the lowest concentration of essential oil inhibiting visible growth of the bacteria [10]. The MICs of *Mentha longifolia* and *Rosmarinus*

*officinalis* essential oils were conventionally determined in triplicate for each strain by the macrodilution broth method as described by Gibbons and Udo [11]. Serial dilutions of both essential oil were prepared in macrodilution tubes with concentrations ranging between (1/2)156.25µg/ml and (1/128) 20,000 µg/ml (Fig. 1). Bacterial suspensions were adjusted to the logarithmic-phase growth to match the turbidity of a 0.5 McFarland standard, yielding approximately $10^6$ CFU/mL. The same amounts of bacteria were added to all tubes and the tubes were incubated at 37ºC for 24 h. Each tube was examined for growth and was compared with the control. Without adding bacteria, medium with no essential oil, medium containing DMSO and different essential oil concentrations were used as control for each mentioned components, respectively. The absence of growth was defined as an antibacterial activity. Bacterial inoculum was prepared by suspension of freshly grown bacteria in sterile saline (0.85% NaCl w/v) [12] and was adjusted to a 0.5 McFarland standard. The MBC is the lowest concentration of antibiotic required for killing a particular bacterium [13]. The eight dilutions were run in duplicate for the MBC test. At the end of 24 h of incubation, the tubes were read for the MIC and then the MBC by spectrophotometrical method using ELISA READER (Bio-Tek Instruments) at 580 nm.

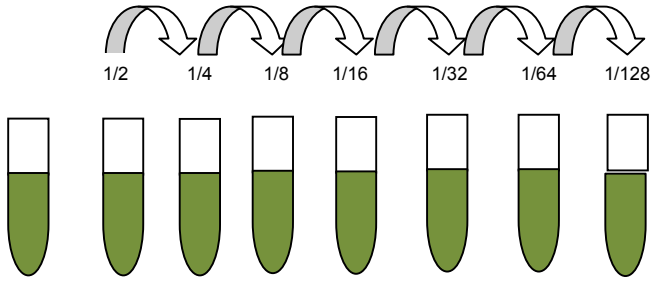


**Fig. 1. Macrodilution tube method for the MIC assay and the MBC assay**

## 3. RESULTS AND DISCUSSION

Different antibacterial properties of essential oils depend on their chemical composition, which varies depending on species and growing conditions of medicinal plants. Based on the dry weight of the plant, the yield of rosemary essential oil obtained by the steam distillation was 1.3% (w/w) in Zabol. The major composition of the rosemary essential oil was alpha-Ocimene (12.31%), 1, 8- Cineole (10.51%), Borneol (8.93%), Geraniol (8.27%), and Camphor (7.75%). The approximate values of other compounds within the rosemary essential oil are below 5% as shown in Table 1 where all components are listed according to their retention time on the DB-5 column. GC/MS analysis has allowed the identification of nineteen components in *Rosmarinus officinalis*, accounting for 97.5% of essential oil (Table 1).

**Table 1. Chemical composition of *Rosmarinus officinalis* essential oil**

| No | Compounds | % | Rt (min) | Type of Compounds |
|---|---|---|---|---|
| 1 | Alpha- Pinene | 7.68 | 14.756 | monoterpene hydrocarbons |
| 2 | Camphene | 2.87 | 15.658 | monoterpene hydrocarbons |
| 3 | 2-beta- Pinene | 4.73 | 16.595 | monoterpene hydrocarbons |
| 4 | 1,8- Cineole | 10.59 | 18.164 | oxygenated monoterpenes |
| 5 | Gamma- Terpinene | 1.69 | 19.262 | oxygenated monoterpenes |
| 6 | Alpha- Terpinolene | 4.53 | 20.145 | oxygenated monoterpenes |
| 7 | Linalool | 7.51 | 20.681 | oxygenated monoterpenes |
| 8 | Camphor | 7.75 | 22.539 | oxygenated monoterpenes |
| 9 | Borneol | 8.93 | 23.018 | oxygenated monoterpenes |
| 10 | Isoborneol | 4.41 | 23.870 | oxygenated monoterpenes |
| 11 | Alpha-Ocimene | 12.31 | 24.37 | monoterpene hydrocarbons |
| 12 | Geraniol | 8.27 | 25.851 | oxygenated monoterpenes |
| 13 | Iso-bornyl acetate | 5.21 | 26.611 | monoterpene hydrocarbons |
| 14 | 1,5,5-Trimethyl-6-methylene-cyclohexene | 1.13 | 27.528 | monoterpene hydrocarbons |
| 15 | Geranyl acetate | 2.98 | 28.608 | oxygenated monoterpenes |
| 16 | Methyleugenol | 2.46 | 29.313 | phenylpropanoid |
| 17 | Trans-Caryophyllene | 2.69 | 30.223 | sesquiterpene hydrocarbons |
| 18 | Alpha-Humulene | 0.48 | 30.986 | sesquiterpene hydrocarbons |
| 19 | Caryophyllene oxide | 1.28 | 34.221 | oxygenated sesquiterpenes |
| **Total identified** | | **97.5** | | |

*Compounds were listed in order of retention time relative to C8–C22 n-alkanes on the DB-5 MS capillary column.*

** Rt=Retention time. In gas chromatography, retention time is the time at which the maximum of a symmetrical peak occurs on a gas chromatogram.*

Based on the dry weight of the plant, the hydrodistillation of aerial parts of *Mentha longifolia* L. gave oil in 1.9% (w/w) yield in Zabol. Thirty-two components accounting to 94.27% of the total oil were identified. The qualitative and quantitative essential oil compositions are presented in Table 2, where compounds are listed in order of their retention time on the DB-5 column. GC-MS analysis revealed that *M. longifolia* was constituted by pulegone (22.37%), Isomenthone (11.56%), beta- Pinene (7.02%), Piperitenone oxide (6.75%) and 1, 8- Cineole (6.60%). The approximate values of other compounds within the Mint essential oil are below 7% as shown in Table 2.

**Table 2. Chemical Composition of *Mentha longifolia* L. essential oil**

| No | Compounds | % | Rt (min) | Type of compounds |
|---|---|---|---|---|
| 1 | Alpha-Thujene | 0.38 | 14.355 | monoterpene hydrocarbons |
| 2 | Alpha- Pinene | 3.06 | 14.713 | monoterpene hydrocarbons |
| 3 | Camphene | 0.34 | 15.237 | monoterpene hydrocarbons |
| 4 | Beta- Pinene | 7.02 | 16.321 | monoterpene hydrocarbons |
| 5 | 1,8- Cineole | 6.60 | 18.079 | oxygenated monoterpenes |
| 6 | Gamma- Terpinene | 1.08 | 18.923 | monoterpene hydrocarbon |
| 7 | 3,8- p- Menthadiene | 0.37 | 19.270 | oxygenated monoterpenes |
| 8 | Alpha- Terpinolene | 0.21 | 19.872 | oxygenated monoterpenes |
| 9 | Isomenthone | 11.56 | 22.462 | oxygenated monoterpenes |
| 10 | Cis-p-Menthan-3-one | 6.65 | 23.168 | oxygenated monoterpenes |
| 11 | (+)-Neomenthol | 5.83 | 23.391 | oxygenated monoterpenes |
| 12 | L-(-)-Menthol | 2.42 | 24.371 | oxygenated monoterpenes |
| 13 | Pulegone | 22.37 | 24.833 | oxygenated monoterpenes |
| 14 | Piperitone | 1.80 | 26.441 | oxygenated monoterpenes |
| 15 | 3-p-Menthene | 1.36 | 26.715 | oxygenated monoterpenes |
| 16 | p-Cymen-3-ol | 1.16 | 27.092 | oxygenated monoterpenes |
| 17 | Carvone | 3.56 | 27.505 | oxygenated sesquiterpenes |
| 18 | Piperitenone oxide | 6.75 | 28.145 | oxygenated monoterpenes |
| 19 | Piperitenone | 4.96 | 28.507 | oxygenated monoterpenes |
| 20 | Alpha-Capaene | 0.24 | 29.020 | Sesquiterpene hydrocarbone |
| 21 | Beta- Gurjunene | 0.86 | 29.294 | Sesquiterpene hydrocarbone |
| 22 | Trans-Caryophyllene | 0.36 | 30.150 | oxygenated sesquiterpenes |
| 23 | Germacrene-D | 0.26 | 30.674 | sesquiterpene hydrocarbons |
| 24 | Carvenone | 0.64 | 31.025 | oxygenated sesquiterpenes |
| 25 | Beta- Cubebene | 1.76 | 31.646 | sesquiterpene hydrocarbons |
| 26 | Bicyclogermacrene | 0.53 | 31.973 | sesquiterpene hydrocarbons |
| 27 | Delta- Cadinene | 0.22 | 32.405 | sesquiterpene hydrocarbons |
| 28 | Cis- alpha- Bisabolene | 0.54 | 32.652 | sesquiterpene hydrocarbons |
| 29 | Spathulenol | 0.76 | 33.928 | oxygenated Sesquiterpene |
| 30 | Cis- alpha- Copaene-8-ol | 0.31 | 34.610 | oxygenated Sesquiterpene |
| 31 | Methyl jasmonate | 0.13 | 35.112 | oxygenated monoterpenes |
| 32 | Isospathulenol | 0.18 | 35.243 | oxygenated Sesquiterpene |
| **Total identified** | | **94.27** | | |

*Compounds listed in order of retention time relative to C8–C22 n-alkanes on the DB-5 MS capillary column.*

**Rt=Retention time. In gas chromatography, retention time is the time at which the maximum, of a symmetrical peak occurs on a gas chromatogram.*

The antibacterial activities of mint and rosemary oils are summarized in Table 3. The results of the antibacterial activity assays indicated that the essential oil of mint exhibited higher activity against the *Listeria monocytogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*. Both oils had the same effect on *E. coli* and the inhibitory effect was non-significant. Activity of rosemary oil against *Salmonella typhi* and *Bacillus licheniformis* was higher than mint oil.

In general, *E. coli* is the most resistant strain and needs high concentrations of essential oils for inhibitory action (Table 3).

**Table 3. Antibacterial Power of *Mentha longifolia* L. and *Rosmarinus officinalis***

| Bacteria | Mint Essential Oil | | Rosemary Essential Oil | |
|---|---|---|---|---|
| | MIC (µg/ml) | MBC (µg/ml) | MIC (µg/ml) | MBC (µg/ml) |
| *Escherichia coli* (PTCC 1533) | 20000 | 20000 | 20000 | 20000 |
| *L. monocytogenes* (PTCC 1163) | 5000 | 5000 | 10000 | 10000 |
| *Bacillus cereus* (ATCC 11778) | 625 | 625 | 20000 | 20000 |
| *Bacillus licheniformis* (PTCC 1525) | 10000 | 10000 | 2500 | 2500 |
| *Pseudomonas aeruginosa* (ISIRI 275) | 156.25 | 156.25 | 625 | 625 |
| *Staphylococcus aureus*(PTCC 1112) | 156.25 | 156.25 | 312.5 | 312.5 |
| *Salmonella* Typhi(PTCC 1609) | 20000 | 20000 | 1250 | 1250 |

*MIC= Minimum Inhibitory Concentration: i.e., the lowest concentration of a particular antibiotic needed to kill bacteria.*

*MBC= Minimum Bactericidal concentration; i.e., the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by ≥99.9%.*

*M. longifolia* essential oils from other geographical locations have been extensively studied. The essential oil content (1.9% v/w in dry leaf) was in accordance with the earlier published data [14,15]. In the extracted oil of *M. longifolia* collected in the flowering stage, the oxygenated monoterpenes were found to be the major class of substances (75.73%), followed by monoterpene hydrocarbons (11.88%), sesquiterpene hydrocarbons (4.41%) and oxygenated sesquiterpenes (2.25%).These results are in accordance with the previously published data except limonene compound which was not found in the sample of Zabol. Main constituents in *Mentha longifolia* samples collected from various locations were Tunisia, pulegone (54.41%) was followed by isomenthone (12.02%), 1, 8-cineole (7.41%), borneol (6.85%), and piperitenone oxide (3.19%) [14], Tajikistan, Cis-piperitone epoxide (7.8-77.6%), piperitenone oxide (1.5-49.1%), carvone (0.0-21.5%), menthone (0.0-16.6%), thymol (1.5-4.2%), pulegone (0.3-5.4%), β-thujone (0.2-3.2%),(E)-caryophyllene (0.9-2.5%), myrcene (0.3-2.5%), carvacrol (0.0-2.7%), borneol (0.9-1.8%), and p-cymene (0.2-1.9%) [15]As a major component, Iran, piperitone (44%), limonene (14%), and trans-piperitone (13%) [16].

In the oil obtained from the *Rosmarinus officinalis* and collected in the flowering stage, the oxygenated monoterpenes were found to be the major class of substances (56.66%), followed by the monoterpene hydrocarbons (33.93%), sesquiterpene hydrocarbons (3.17%), oxygenated sesquiterpenes (1.28%) and phenylpropanoid (2.46%).

These results are in accordance with the previously published data except Verbenone, which was not found at all. Other components were found in low concentration in *Rosmarinus officinalis* essential oil. Main constituents in *Rosmarinus officinalis* samples collected from various locations were South Africa, Camphor (32.12%), 1, 8-Cineole (31.12%), α-Pinene

(18.18%), Verbenone (4.12%) and β-Pinene (2.58%) [17,18]; *Iran*, α-pinene (14.9%), 1, 8-cineole (7.43%) and linalool (14.9%) [19].

Significant antibacterial activity of essential oil was recorded against that of the examined multi resistant pathogenic bacteria, *E. coli*, *Pseudomonas aeruginosa, Salmonella typhi, Listeria monocytogenes*, *Bacillus cereus*, *Bacillus licheniformis* and *Staphylococcus aureus*. Especially considerable is that the highest sensitivity to essential oil of *M. longifolia* and *Rosmarinus officinalis* was observed in *Pseudomonas aeruginosa* and *Staphylococcus aureus.* In conclusion, the study revealed significant antibacterial activity of the investigated essential oils. The examined oil exhibited high resistant pathogenic bacteria, which was found to be in correlation to the content of mainly monoterpene ketones and aldehydes. These results indicate that essential oils could be served not only as flavor agents but also as safe antiseptic supplements in preventing deterioration of foodstuff, beverage products, and pharmaceuticals.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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