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**ORIGINAL ARTICLE**

# Chemical Composition and Antimicrobial Activity of Essential Oil of *Matricaria chamomilla*

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**ABSTRACT**
*The present study describes the phytochemical profile and antimicrobial activity of Matricaria chamomilla essential oil. The sample of essential oil was obtained from the aerial parts of the plant by hydrodistillation and analyzed by GC–MS. A total 55 compounds were identified mainly including α-Pinene (22.11%), Camphene (10.8%), Sabinene (4%), Limonene(5.64%),1,8-Cineole(6.45%), Camphor (4%) and α-Bisabolol(6.35%). Essential oil of Chamomile was evaluated for its antibacterial activities against three Gram-positive and four Gram negative pathogenic bacteria: Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Proteus sp., Pseudomonua aeruginosa, Shigella sonnei and Shigella shiga. Antimicrobial activity of the oil was evaluated using agar diffusion and micro dilution methods. The antimicrobial test results showed that the oil had antimicrobial activity against all bacteria strains, except Pseudomonas aeruginosa. Results, suggest antimicrobial activities had correlated to the chemical composition.*
***Key Words**: Matricaria chamomilla, antimicrobial activity; Essential oil*

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## INTRODUCTION

*Matricaria chamomilla* (Chamomile) is one of the important medicinal herb native to Iran. Chamomile, in particular their flower-head contained several groups of compounds having important therapeutic values especially sesquiterpene essential oil [1]. The terpenes, α-bisabolol oxides and chamazulene, are the most important compounds [2]. The essential oils (monoterpenes) of a number of plants exhibit specific biological, pharmaceutical and activities [3]. Essential oils extracted from plants could be employed as antimicrobial agents [4]. Prabuseenivasan *et al.,* [5] showed that essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens. Recent studies have shown that Chamomile species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities [6]. Lu *et al.,* [7] reported the antibacterial activity of six matricaria esters against *Mycobacterium tuberculosis* and *Mycobacterium avium*, using a radiorespirometric bioassay. Pauli [8] reported that α-bisabolol from *Matricaria chamomilla* may inhibit fungal growth via specific inhibition of ergosterol biosynthesis. The aim of the present work was to determine the essential oil composition of *Matricaria chamomilla* and to evaluate its antimicrobial activity against human pathogenic.

## MATERIALS AND METHODS

**Plant material:** Samples of *Matricaria chamomilla* were collected in western Iran in season 2012-2013. Then the plants were isolated from the other specimen and conserved for extraction. The essential oils were extracted by hydro distillation using an apparatus of Clevenger. For this, 500 g of plants was used in 1600 ml of distilled water the extraction took 3 hours. After filtration the solvent is eliminated by reduced pressure distillation in rotary evaporator and pure oil was stored at 4°C in obscurity till the beginning of analysis. GC analysis was performed, using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was held at 50°C for 5 min and then programmed to 250°C at a rate of 3°C/min. Injector and detector (FID) temperatures were 290°C; helium was used as carrier gas with a linear velocity of 32 cm/s. The percentages were

calculated by electronic integration of FID peak a reas without the use of response factors correction. Linear retention indices for all components were determined by co injection of the samples with a solution containing homologous series of $C_8$-$C_{22}$ n-alkanes. GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.); oven temperature was 40°C to 240 °C at a rate of 4°C. Transfer line temperature was 260°C. Carrier gas was helium with a linear velocity of 31.5 cm/s, split ratio 1/60. In addition, ionization energy was 70 eV, scan time 1 s, and mass range 40-300 amu. Identification of components in the oil was based on retention indices relatives to n-alkanes and computer matching with the WILLEY 275. L library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature [1]. The chromatographic conditions were identical to those used for GC analysis.

**Tests for antibacterial activity**

The microorganisms used in the present study were three Gram positive (*Staphylococcus aureus, Bacillus cereus,Bacillus subtilis*) and four Gram negative (*Shigella shiga, Shigella sonnei, Pseudomonua aeruginosa* and *Proteus sp*.) human pathogenic bacteria.

**Disc-diffusion test**

Compounds were investigated by the disc diffusion using 4 mm filter discs. Bacteria were cultured overnight at 28°C in LB medium and then adjusted with sterile saline to a concentration of 1.0 × 105 cfu/ml. The suspension was added to the top of agar (6 ml) and dissolved in Petri dishes (2 ml/agar plate) with solid peptone agar. Filter discs with essential oils and main components (1.0 µg/ ml) were placed on agar plates (1 disc per agar plate). After 24 h of incubation at 28°C for bacteria the diameter of the growth inhibition zones was measured. Streptomycin was used as a positive control, and 1 µl was applied to the discs from stock solution (1 mg/ml). All tests were done in duplicate; three replications were done for each oil and for each component [9].

**Microdilution test**

The minimum inhibitory and bactericidal and fungicidal concentrations (MICs and MBCs) were determined using microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0 x 105 cfu/ml. Compounds to be investigated were dissolved in broth LB medium (100 µl) with bacterial inoculum (1.0 × 104 cfu per well) to achieve the wanted concentrations (0.02-15.0 µg/ml). The microplates were incubated for 24 h at 28°C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial sub-cultivation of 2 µl into microtitre plates containing 100 µl of broth per well and further incubation for 72 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank and the positive control. Streptomycin was used as a positive control using the same concentrations as in the disc diffusion test. Three replications were done for each oil and each component [9].

## RESULTS AND DISCUSSION

The results obtained by GC–MS analyses of the essential oil of *Matricaria chamomilla* are presented in Table 1. Thirty seven compounds were identified in the essential oil. As a result of GC–MS analyse, *Matricaria chamomilla* contained, α-Pinene (22.11%), Camphene (10.8%), Sabinene (4%), Limonene(5.64%),1,8-Cineole(6.45%), Camphor (4%) and α-Bisabolol(6.35%). as the major compounds. Other significant constituents were d-3-Carene (0.13%), α-Terpinene (1.6%), p-Cymene (0.31%), β-Phellandrene (0.35%), Benzeneacetaldehyde (0.36%), γ-Terpinene (1%), Artemisiaketone (0.34%), Z-Sabinenehydrate (0.87%), α-Linalool (0.22%), α-Thujone (0.34%), β-Thujone (0.45%), E-Sabinol (0.89%), Borneol (0.65%), 4-Terpineol (0.47%), α-Terpineol (0.69%), E-Piperitol (0.48%), α-Terpinylacetate (0.22%), α-Cubebene (0.47%),α-Isocomene (0.36%),β-Elemene (0.4%),α-Funebrene (0.65%), Isocaryophyllene (0.65%), β-Caryophyllene(0.5%),E-β-farnesene (0.68%) ,GermacreneD (0.9%), Bicyclogermacrene (0.85%), E-Nerolidol (0.28%), Spathulenol (0.76%), Caryophyllene oxide (0.63%), Bisabolol oxide B(0.45%) and α-Bisabolol (6.35%). The steam distillation of 500 g of the air-dried plant yielded 3.5 mL oil. The results indicate the essential oil from *Matricaria chamomilla* share some relatively similar main constituents, such as matricaria ester, farnesene and bisabolol, with other several species of *Matricaria* and serve as chemosystematic markers of *M. songarica* [10-12]. Table 2 presents the inhibition zone of essential oil determined for 4 of Gram positive or Gram negative bacteria using the diffusion technique. The results showed that the essential oil had a substantial inhibitory effect on all assayed bacteria strains noted by large growth inhibition halos. The result indicated that Gram-positive *Staphylococcus aureus* was the most sensitive strain tested to the oil of *Matricaria chamomilla*

with the strongest inhibition zone (35 mm). The oil also exhibited high antimicrobial activity against *Bacillus cereus* and *Bacillus subtilis* (30 and 28 inhibition zone, respectivly ). Among these, Gram negative strains also displayed variable degree of susceptibility against investigated oil. Maximum activity was observed against *Shigella shiga* and *Shigella sonnei* with inhibition zone of 20 mm. while *Matricaria chamomilla* essential oil showed lower antimicrobial activity against *Pseudomonua aeruginosa* and *Proteus sp.* than that of standard streptomycin (Table 2). The results of the MIC and MBC are presented in Table III. As shown in Table 3, streptomycin exhibited highest antimicrobial effect against all employed microorganisms except *Pseudomonua aeruginosa.* The data indicate that the oil exhibited varying levels of antimicrobial activity against the investigated human pathogens. MIC values showed by the essential oil were in the range of 0.021 to 6 µg /mL. MBC values showed by the essential oil were in the range of 0.5 to 10 µg /mL. The Gram negative *Pseudomonua aeruginosa* resistant to the investigated oil with a MIC of 6 µg /mL and MBC of 10 µg /mL. Maximum activity was observed against the *Staphylococcus aureus, Bacillus cereus and Bacillus subtilis* with a MIC of 0.021, 0.032 and 0.041 µg /mL, respectivly. *Pseudomonua aeruginosa* was the least sensitive bacteria with MBC of 10 µg /mL. *Shigella shiga*, *Shigella sonnei* and *Proteus* sp. showed similar susceptibility to the investigated oil, 5 µg/Ml (MBC). The results of antibacterial activity of essential oil components are presented in Tables 4 and 5. Limonene (inhibition zones: 5.0-18.0 mm), Camphor (inhibition zones: 8.0-20.0 mm) and Sabinene (inhibition zones: 10.0-15.0 mm) showed the lowest antibacterial activity among the components tested. 1,8-Cineole inhibited bacterial growth of all bacteria and inhibition zones were 14.0-28.0 mm, while Streptomycin showed activity with inhibition zones 10.0-25.0 mm. Bisabolol and Bisabolol oxide showed inhibition with zones of 15.0-30.0 mm. Limonene, Camphor and Sabinene showed the lowest antibacterial activity in the microdilution method, MIC at 5.0-10.0 µg/ml and MBC at 4.0-12.0 µg/ml. Camphene and 1,8-Cineole exhibited inhibitory activity at 3.0-8.0 µg/ml and was bactericidal at 4.0-8.0 µg/ml, while, Streptomycin exhibited inhibitory activity at 5.0-10.0 µg/ml and was bactericidal at 4.0-8.0 µg/ml . Among the eight essential oil components tested, Bisabolol (MIC at 0.5-6.0 µg/ml and MBC at 0.5-2.0 µg/ml) and carvacrol (MIC at 1.0-1.5 µg/ml and MBC at 0.5-1.5 µg/ml) showed the highest activity. According to our results, Gram-positive bacteria were more susceptible than Gram-negative bacteria to the antimicrobial activity of essential oil, which is in accord with to some previous reports [13-15]. Significant difference was observed between Gram positive and Gram negative bacteria in terms of their
susceptibility, so that Gram positive bacteria were more sensitive to antimicrobial activity of feverfew essential oil. The higher sensitivity of Gram positive bacteria may be explained according to their cell wall structure. Imelouane *et al.,* [16] observed that the susceptibility of Gram positive and Gram negative bacteria to plant volatile oils had a little influence on growth inhibition. The cell wall structure of Gram negative bacteria is constituted essentially with Lipopolysaccharides. This constituent avoids the accumulation of the oils on the cell membrane [17]. The antimicrobial activity of the essential oil from *chamomilla* may be associated with its major components such farnesene, bisabolol oxide, bisabolol, matricaria ester, and farnesol. There were, however, significant differences between main components. For example, the major constituent of the *Matricaria chamomilla* oil in our research α-Pinene, Camphor, Sabinene and 1, 8-cineole, was also reported to be the major at the major constituent in our sample.These changes in the essential oil compositions might arise from several environmental (climatical, seasonal, geographical) and genetic differences[18]. Maria-Rose *et al.,* [19] claimed that farnesene have enormous potential against food spoilage and foodborne pathogenic bacteria. Pauli [8] reported that α-bisabolol from *Matricaria chamomilla* may inhibit fungal growth via specific inhibition of ergosterol biosynthesis. Togashi *et al.,* [20] reported the antibacterial activity of farnesol against *Staphylococcus aureus*. *Pseudomonua aeruginosa* was the only bacterium that was not susceptible to the oil, since it is known to have high level of intrinsic resistance to virtually all known antimicrobials and antibiotics due to a combination of a very restrictive outer membrane barrier, highly resistant even to synthetic drugs [21]. The tolerance of Gram negative bacteria to essential oil has been ascribed to the presence of a hydrophilic outer membrane that blocks the penetration of hydrophobic essential oils into target cell membrane. However, some oils appeared more active with respect to Gram reaction, exerting a greater inhibitory activity against Gram-positive bacteria. It was often reported that Gram-negative bacteria were more resistant to the essential oils present in plants [22]. In our study, the antimicrobial activity of the oil could be due to α-Pinene, Camphene, Sabinene, 1,8-Cineole, Bisabolol oxide and α-Bisabolol. The antimicrobial activity of the essential oil of *Matricaria chamomilla* is apparently related to its terpenes type components. The essential oils containing terpenes are also reported to possess antimicrobial activity [23] (Dorman and Deans 2000), which are consistent with our present studies. Iran is one of the richest countries of the world in terms of having a substantial number of different medicinal plants species grown in various ecological conditions. In conclusion, essential oil of Chamomile showed

significant antimicrobial activity. α-Pinene, Camphene, Sabinene, 1,8-Cineole, Bisabolol oxide and α-Bisabolol were common in all the oils as six major compounds. The results suggest that Chamomile essential oils possess some compounds with antimicrobial properties, which can be used as antimicrobial agents in drugs for treatment of infectious diseases. Further researches are needed to get more information on safety and toxicity of this oil.

**Table 1: Chemical composition of essential oil of *Matricaria chamomilla***

| Peak no. | Components | Chamomile % | Identification methods |
|---|---|---|---|
| 1 | α-Pinene | 22.11 | MS,RI |
| 2 | Camphene | 10.08 | MS,RI |
| 3 | Sabinene | 4 | MS,RI |
| 4 | d-3-Carene | 0.03 | MS,RI |
| 5 | α-Terpinene | 1.6 | MS,RI |
| 6 | p-Cymene | 0.31 | MS,RI |
| 7 | β-Phellandrene | 0.35 | MS,RI |
| 8 | Limonene | 5.64 | MS,RI |
| 9 | 1,8-Cineole | 3.45 | MS,RI |
| 10 | Benzeneacetaldehyde | 0.36 | MS,RI |
| 11 | γ-Terpinene | 1 | MS,RI |
| 12 | Artemisiaketone | 0.34 | MS,RI |
| 13 | Z-Sabinenehydrate | 0.87 | MS,RI |
| 14 | α-Linalool | 0.22 | MS,RI |
| 15 | α-Thujone | 0.34 | MS,RI |
| 16 | β-Thujone | 0.45 | MS,RI,Co |
| 17 | E-Sabinol | 0.89 | MS,RI,Co |
| 18 | Camphor | 4 | MS,RI |
| 19 | Borneol | 0.65 | MS,RI |
| 20 | 4-Terpineol | 0.74 | MS,RI |
| 21 | α-Terpineol | 0.69 | MS,RI,Co |
| 22 | E-Piperitol | 0.48 | MS,RI,Co |
| 23 | α-Cubebene | 0.47 | MS,RI |
| 24 | α-Terpinylacetate | 0.22 | MS,RI |
| 25 | α-Isocomene | 0.36 | MS,RI |
| 26 | β-Elemene | 0.4 | MS,RI |
| 27 | α-Funebrene | 0.65 | MS,RI |
| 28 | Isocaryophyllene | 0.56 | MS,RI,Co |
| 29 | β-Caryophyllene | 0.5 | MS,RI |
| 30 | E-β-farnesene | 0.68 | MS,RI,Co |
| 31 | GermacreneD | 0.9 | MS,RI |
| 32 | Bicyclogermacrene | 0.85 | MS,RI |
| 33 | E-Nerolidol | 0.28 | MS,RI |
| 34 | Spathulenol | 0.76 | MS,RI |
| 35 | Caryophyllene oxide | 0.63 | MS,RI |
| 36 | Bisabolol oxide | 8.45 | MS,RI |
| 37 | α-Bisabolol | 6.35 | MS,RI,Co |
| | Total | 80.76 | |

**Table2. Antibacterial activity of essential oils (1.0 µg/ml) in disc-diffusion method, inhibition zones in mm.**

| microorganisms | Chamomile % | streptomycin |
|---|---|---|
| **Gram positive** | | |
| ***Staphylococcus aureus*** | **35** | **20** |
| ***Bacillus cereus*** | **30** | **20** |
| ***Bacillus subtilis*** | **28** | **20** |
| **Gram negative** | | |
| ***Shigella shiga*** | **20** | **18** |
| ***Shigella sonnei*** | **20** | **18** |
| ***Pseudomonua aeruginosa*** | **16** | **18** |
| ***Proteus* sp.** | **15** | **16** |

**Diameter of inhibition zones (mm) including the diameter of disc (6 mm)**

**Table 3. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of essential oil from Lavandula dentata**

| microorganisms | Essential oil Chamomile | | streptomycin | |
|---|---|---|---|---|
| **Gram positive** | **MIC** | **MBC** | **MIC** | **MBC** |
| ***Staphylococcus aureus*** | **0.021** | **0.5** | **0.145** | **1** |
| ***Bacillus cereus*** | **0.032** | **1** | **0.135** | **1.5** |
| ***Bacillus subtilis*** | **0.041** | **1** | **0.130** | **1** |
| **Gram negative** | | | | |
| ***Shigella shiga*** | **0.178** | **5** | **0.125** | **3** |
| ***Shigella sonnei*** | **0.195** | **5** | **0.2** | **2** |
| ***Pseudomonua aeruginosa*** | **6** | **10** | **3** | **6** |
| ***Proteus* sp.** | **0.161** | **5** | **0.2** | **3** |

**MIC:Minimum inhibitory concentration (values in µg/ml)**
**MBC: Minimum bactericidal concentration (values in µg/ml)**

**Table 4 Antibacterial activity of essential oils components (1.0 µg/ml) in disc-diffusion method, inhibition zones in mm.**

| microorganisms | α-Pinene | Camphene | Sabinene | Limonene | 1,8-Cineole | Camphor | Bisabolol | Bisabolol oxide | streptomycin |
|---|---|---|---|---|---|---|---|---|---|
| Gram positive | | | | | | | | | |
| *Staphylococcus aureus* | 22 | 25 | 15 | 10 | 28 | 20 | 30 | 30 | 25 |
| *Bacillus cereus* | 25 | 21 | 15 | 10 | 25 | 15 | 32 | 32 | 22 |
| *Bacillus subtilis* | 22 | 20 | 20 | 10 | 25 | 20 | 28 | 35 | 25 |
| Gram negative | | | | | | | | | |
| *Shigella shiga* | 10 | 16 | 12 | 5 | 15 | 10 | 20 | 20 | 10 |
| *Shigella sonnei* | 15 | 15 | 15 | 5 | 10 | 9 | 14 | 22 | 9 |
| *Pseudomonua aeruginosa* | 15 | 12 | 10 | 5 | 15 | 15 | 10 | 20 | 10 |
| *Proteus* sp. | 15 | 15 | 10 | 5 | 14 | 8 | 10 | 15 | 10 |

**Diameter of inhibition zones (mm) including the diameter of disc (6 mm)**

**Table 5 Antibacterial activity of essential oils components (MIC and MBC - µg/ml), microdilution method**

| microorganisms | α-Pinene | | Camphene | | Sabinene | | Limonene | | 1,8-Cineole | | Camphor | | Bisabolol | | Bisabolol oxide | | streptomycin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gram positive | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| *Staphylococcus aureus* | 5 | 5 | 3 | 5 | 6 | 6 | 5 | 4 | 6 | 4 | 3 | 3 | 2.5 | 1 | 0.5 | 0.5 | 5 | 5 |
| *Bacillus cereus* | 6 | 6 | 5 | 4 | 6 | 5 | 6 | 4 | 4 | 5 | 4 | 5 | 1 | 1.5 | 1 | 0.5 | 5 | 4 |
| *Bacillus subtilis* | 5 | 4 | 5 | 5 | 5 | 4 | 5 | 4 | 4 | 5 | 3 | 2 | 0.5 | 0.5 | 0.5 | 1 | 5 | 7 |
| Gram negative | | | | | | | | | | | | | | | | | | |
| *Shigella shiga* | 7 | 8 | 6 | 8 | 8 | 5 | 7 | 8 | 6 | 8 | 6 | 6 | 3 | 2 | 2 | 1 | 8 | 8 |
| *Shigella sonnei* | 7 | 4 | 4 | 8 | 5 | 4 | 7 | 4 | 7 | 5 | 7 | 5 | 5 | 3 | 4 | 1.5 | 6 | 7 |
| *Pseudomonua aeruginosa* | 10 | 12 | 8 | 8 | 10 | 10 | 10 | 12 | 7 | 8 | 6 | 8 | 6 | 2 | 5 | 1.5 | 10 | 8 |
| *Proteus* sp. | 9 | 5 | 8 | 8 | 8 | 12 | 7 | 10 | 8 | 8 | 5 | 8 | 5 | 2 | 3 | 1 | 8 | 8 |

**MIC:Minimum inhibitory concentration (values in µg/ml)**
**MBC: Minimum bactericidal concentration (values in µg/ml)**

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