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Chemical Composition and Antimicrobial Activity of Essential Oil of *Matricaria recutita*

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Matricaria recutita is a herbaceous plant belonging to the Asteraceae family. The present study reports the chemical composition and antimicrobial activity of *M. recutita* essential oil and its main compounds. The essential oil was obtained from the aerial parts of the *M. recutita* by hydrodistillation and analyzed by gas chromatography-mass spectrometry. The major components were α-bisabolol oxide (38%), followed by camphene (9.11%), sabinene (4.87%), limonene (6%),1,8-cineole (7.12%), camphor (6.54%), and α-pinene (6%). Essential oil of chamomile was evaluated for its antibacterial activities against three gram-positive and four gram-negative pathogenic bacteria. The essential oil and its main compounds were particularly active against *Bacillus cereus,* with the lowest minimum inhibitory concentration and minimum bactericidal concentration value (0.022 and 1.5 μg /mL). In conclusion, these results support the use of the essential oil and its main compounds for their antimicrobial properties.

Keywords: *Matricaria recutita*, Antimicrobial activity, Essential oil, GC/MS.

INTRODUCTION

Plants are rich sources of beneficial secondary metabolites. Their essential oils have a wide array of biological activities, especially antimicrobial effects.[1,2,3] Chamomile (*Matricaria recutita* L.) is a medicinal plant that contains a large number of therapeutic and active compounds. *M. recutita* is being cultivated commercially as a medicinal herb with several applications in traditional medicine in different parts of Iran. Potentially active chemical constituents of *M. recutita* including terpenoids and flavonoids spiroethers which are believed to be responsible in part for such a wide range of biological activities.[4] The main biologically active compounds in chamomile oil have been found to be bisabolol oxides, bisabolone oxide, a-bisabolol, spathulenol, enyne-dicycloethers, and chamazulene.[5,6] *Matricaria recutita* was evaluated for neuroprotective,[7] anti-inflammatory, antiseptic, and spasmolytic properties.[8] The aim of the present work was to determine the essential oil composition of *Matricaria recutita* and to evaluate its antimicrobial activity against human pathogenic.

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MATERIALS AND METHODS

Plant Material and Oil Isolation

The aerial parts of wild growing *Matricaria recutita* were gathered during the flowering period in 2012–2013 from the regions of central Iran. The *Matricaria recutita* aerial parts were ground and the resulting powder was subjected to hydrodistillation for 3 h in an all glass Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia.[9] The obtained essential oils were dried over anhydrous sodium sulphate and after filtration, stored at +4°C until tested and analyzed.

Essential Oil Analysis

The gas chromatography-mass spectrometry (GC/MS) analyses were executed on a Hewlett-Packard 5973N gas chromatograph equipped with a column HP-5MS (30 m length × 0.25 mm i.d., film thickness 0.25 lm) coupled with a Hewlett-Packard 5973N mass spectrometer. The column temperature was programmed at 50°C as an initial temperature, holding for 6 min, with 3°C increases per minute to the temperature of 240°C, followed by a temperature enhancement of 15°C per minute up to 300°C, holding at the mentioned temperature for 3 min. Injector port temperature was 290°C and helium used as carrier gas at a flow rate 1.5 mL/min. Ionization voltage of mass spectrometer in the EI-mode was equal to 70 eV and ionization source temperature was 250°C. Linear retention indices for all components were determined by co-injection of the samples with a solution containing homologous series of C8-C22 *n*-alkanes and comparing them and their mass spectra with those of authentic samples or with available library data of the GC/MS system (WILEY 2001 data software) and Adams libraries spectra.[10]

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 Luria-Bertani (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 (one 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.[11]

Microdilution Test

The minimum inhibitory and bactericidal and fungicidal concentrations minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined using microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0 × 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 component.[11] The quantitative data of major components of oil were statistically examined by one-way analysis of variance (ANOVA), and significant differences among groups were subsequently analyzed by Duncan's multiple range test ($P < 0.05$). Correlation and regression coefficients were performed using Statistical Package for the Social Sciences (SPSS).

RESULTS AND DISCUSSION

Chemical Composition of Essential Oil

The essential oil of Iranian *Matricaria recutita* aerial parts obtained using hydrodistillation was isolated in high yield (0.82%; Table 1). The major constituent of the oil was α-bisabolol oxide, with a relative concentration of 38%. The GC/MS analysis of *Matricaria recutita* oil showed 18 compounds representing 99.06% of the total oil; α-bisabolol oxide was the main constituent (38%) followed by camphene (9.11%), sabinene (4.87%), limonene(6%),1,8-cineole(7.12%), camphor (6.54%), and α-pinene (6%) as the major compounds. Jaimand and Rezaee[12] have analyzed MCEO originating from Kazeroon, Hamedan, and Tehran in Iran. The main components of sample from Kazeroon were as follows: *a*-bisabolol, (*Z, Z*)-farnesol, *cis*-b-farnesene, guaiazulene, and chamazulene (2.60%); in the sample from Hamedan as: (*Z, Z*)-farnesol, *a*-bisabolol oxide B, guaiazulene, and *cis*-b-farnesene; and in the sample from Tehran as: (*Z, Z*)-farnesol, guaiazulene, *a*-bisabolol oxide A, and *cis*-b-farnesene. Our results reinforce previous data on the variability aerial parts volatile oils, depending on the origin of the samples, environmental, and climatic conditions.

Antimicrobial Activity

Table 2 presents the inhibition zone of essential oil determined for four of the 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 results indicated that gram-positive *Bacillus cereus* was the most sensitive strain tested to the oil of *Matricaria recutita* with the strongest inhibition zone (36 mm). The oil also exhibited high antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* (30 and 32 inhibition zone, respectively). Among these, gram-negative strains also displayed variable degree of susceptibility against investigated oil. Maximum activity was observed against *Shigella shiga* with inhibition zone of 25 mm, while *Matricaria recutita* essential oil showed lower antimicrobial activity against *Proteus sp.* than that of standard streptomycin (Table 2). The results of the MIC and MBC are presented in Table 3. The data indicate that the oil exhibited varying levels of antimicrobial activity. MIC values showed by the essential oil were in the range of 0.011 to 4 μg/mL. MBC values showed by the essential oil were in the range of 0.5 to 8 μg/mL. The gram-negative *Pseudomonua aeruginosa* is resistant to the investigated oil with a MIC of 4 μg/mL and MBC of 8 μg/mL. The results of antibacterial activity of essential oil components are presented in Tables 4 and 5. Limonene (inhibition zones: 4.0–12.0 mm), camphor (inhibition zones: 6.0–18.0 mm), and sabinene (inhibition zones:

TABLE 1
Chemical composition of essential oil of *Matricaria recutita*

Peak number	*Components*	*Chamomile %*	*Identification methods*
1	α-Pinene	8	MS,RI
2	Camphene	9.11	MS,RI
3	Sabinene	4.87	MS,RI
4	D-3-Carene	0.1	MS,RI
5	α-Terpinene	2.32	MS,RI
6	P-Cymene	0.5	MS,RI
7	β-Phellandrene	0.9	MS,RI
8	Limonene	6	MS,RI
9	1,8-Cineole	7.14	MS,RI
10	Benzeneacetaldehyde	0.12	MS,RI
11	γ-Terpinene	0.9	MS,RI
12	Artemisiaketone	0.23	MS,RI
13	Z-Sabinenehydrate	0.98	MS,RI
14	α-Linalool	0.12	MS,RI
15	α-Thujone	0.64	MS,RI
16	β-Thujone	0.89	MS,RI,Co
17	E-Sabinol	0.87	MS,RI,Co
18	Camphor	6.54	MS,RI
19	Borneol	0.45	MS,RI
20	4-Terpineol	0.6	MS,RI
21	α-Terpineol	0.61	MS,RI,Co
22	E-Piperitol	0.54	MS,RI,Co
23	α-Cubebene	0.2	MS,RI
24	α-Terpinylacetate	0.7	MS,RI
25	α-Isocomene	0.15	MS,RI
26	β-Elemene	0.78	MS,RI
27	α-Funebrene	0.31	MS,RI
28	Isocaryophyllene	0.3	MS,RI,Co
29	β-Caryophyllene	0.5	MS,RI
30	E-β-farnesene	0.43	MS,RI,Co
31	GermacreneD	0.7	MS,RI
32	Bicyclogermacrene	0.23	MS,RI
33	E-Nerolidol	0.8	MS,RI
34	Spathulenol	0.34	MS,RI
35	Caryophyllene oxide	0.23	MS,RI
36	α-Bisabolol oxide	38	MS,RI
37	α-Bisabolol	1.11	MS,RI,Co
38	Chamazulene	1.31	MS,RI,Co
39	α -Farnesene	1	MS,RI,Co
40	β -Farnesene	1.54	MS,RI,Co
	Yield	0.82	
	Total	80.76	

10.0–20.0 mm) showed the lowest antibacterial activity among the components tested. Bisabolol oxide inhibited bacterial growth of all bacteria and inhibition zones were 12.0–35.0 mm. Sabinene showed the lowest antibacterial activity in the microdilution method, MIC at 5.0–8.0 μg/mL and MBC at 4.0–8.0 μg/mL. Among the eight essential oil components tested, bisabolol oxide (MIC at 0.5–3.0 μg/mL and MBC at 0.5–1.5 μg/mL) showed the highest activity. According to our results,

TABLE 2
Antibacterial activity of essential oils (1.0 μg/mL) in disc-diffusion method, inhibition zones in mm

Microorganisms	*Chamomile %*	*Streptomycin*
Gram positive		
Staphylococcus aureus	30	22
Bacillus cereus	36	22
Bacillus subtilis	32	22
Gram negative		
Shigella shiga	25	17
Shigella sonnei	19	18
Pseudomonua aeruginosa	19	18
Proteus sp.	16	15

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 *Matricaria chamomilla*

	Essential oil chamomile		*Streptomycin*	
Microorganisms	*MIC*	*MBC*	*MIC*	*MBC*
Gram positive				
Staphylococcus aureus	0.011	0.5	0.13	1
Bacillus cereus	0.022	1.5	0.12	1.5
Bacillus subtilis	0.03	1.5	0.115	1
Gram negative				
Shigella shiga	0.14	3	0.11	5
Shigella sonnei	0.2	3	0.1	3
Pseudomonua aeruginosa	4	8	5	8
Proteus sp.	0.15	3	0.1	4

MIC: minimum inhibitory concentration (values in μg/ml); MBC: minimum bactericidal concentration (values in μg/ml); All tests were done in duplicate; three replications were done for oil.

gram-positive bacteria were more susceptible than gram-negative bacteria to the antimicrobial activity of essential oil, which is in accord 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 chamomilla 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. These changes in the essential oil compositions might arise from several environmental (climatical, seasonal, geographical) and genetic differences.[18] Pauli[19] reported that α-bisabolol from *Matricaria chamomilla* may inhibit fungal growth via specific inhibition of ergosterol biosynthesis. 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

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	18	16	14	10	15	20	15	18	15
Bacillus cereus	20	25	20	12	20	22	20	35	20
Bacillus subtilis	20	25	18	12	15	22	15	22	20
Gram negative									
Shigella shiga	12	13	10	5	16	5	15	20	15
Shigella sonnei	14	15	10	5	12	5	15	20	10
Pseudomonua aeruginosa	18	5	10	4	10	15	12	12	10
Proteus sp.	15	15	10	5	10	10	15	20	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

	α-Pinene		*Camphene*		*Sabinene*		*Limonene*		*1,8-Cineole*		*Camphor*		*Bisabolol*		*Bisabolol oxide*		*Streptomycin*	
Microorganisms	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
Gram positive																		
Staphylococcus aureus	5	6	5	5	5	6	5	4	6	5	5	5	1.5	1.5	0.5	0.5	8	7
Bacillus cereus	5	4	5	5	5	5	6	4	5	5	5	4	1.5	1.5	0.5	0.5	6	5
Bacillus subtilis	5	6	5	5	4	4	5	4	5	5	5	4	1	1	0.5	0.5	8	5
Gram negative																		
Shigella shiga	6	6	5	10	6	6	7	8	5	5	10	10	4	4	1	1.5	10	9
Shigella sonnei	7	6	6	10	5	5	7	4	5	6	8	4	4	4	3	1.5	5	9
Pseudomonua aeruginosa	12	12	10	10	8	8	10	12	7	10	5	7	6	4	3	1.5	12	9
Proteus sp.	8	6	10	10	8	8	7	10	10	8	7	8	4	3	3	1.5	5	89

MIC: minimum inhibitory concentration (values in μg/ml); MBC: minimum bactericidal concentration (values in μg/ml);
All tests were done in duplicate; three replications were done for component.

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.[20] 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.

CONCLUSIONS

Our data indicate that the essential oil extracted from *Matricaria recutita* exhibit potent biological activities, which support their use in traditional medicine. Moreover, results regarding the bioactivities of the main volatile components suggest that the observed activities of the essential oil are connected to its chemical composition, where α-bisabolol oxide and bisabolol has been found to be the most active compounds. There was a good correlation between total phenol content and antimicrobial and antioxidant capacity of the extracts. In conclusion, *Matricaria recutita* extracts appear to contain compounds with antimicrobial activities.

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