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Full Length Article

Chemical Composition and Antimicrobial Activities of Essential Oil of *Matricaria songarica*

SU JIAN-YU, L. ZHU[1] AND YING-JUAN TIAN
College of Food and Bioengineering, South China University of Technology, Wushan Road 381, Guangzhou 510641, China
[1]Corresponding author's e-mail: zhuliang@scut.edu.cn

ABSTRACT

Essential oils were obtained by steam distillation from *Matricaria songarica* collected in Gaoligong Mountains, Yunnan Province, China and analyzed by gas chromatography/mass spectroscopy (GC-MS). A total 55 compounds were identified mainly including E-β-farnesene (10.58%), bisabolol oxide A (10.46%), α-bisabolol (9.33%), (Z,Z)-matricaria ester (8.28%), (E,E)-farnesol (8.26%), (E,Z)-matricaria ester (4.05%). Antimicrobial tests showed that the essential oil (1000 μg/disc) exhibited antimicrobial effects against ten tested microorganisms as diameter of zones of inhibition (21.0-23.8 mm) and MIC values (25-200 μg/mL) against *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative), *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive), *Hansenula anomala* and *Saccharomy cescerevisiae* (yeast), *Aspergillus niger*, *Chaetomium globosum*, *Mucor racemosus* and *Monascus anka* (mould). Antimicrobial activities were correlated to the chemical composition.

Key Words: *Matricaria songarica*; Essential oil; Antimicrobial activity; GC-MS

INTRODUCTION

The genus *Matricaria*, classified in subtribe *Matricariinae* of the *Anthemideae*, consists of about 40 species of subshrubs and herbaceous perennials throughout the world. This genus is an important medicinal plant, often used in traditional medicine (Dragland *et al*., 2003), employed as a medicinal tea and major composition of some tinctures. The interest in the essential oils and extracts of *Matricaria* species increased significantly in the past decade, because of their widespread acceptance by consumers, their relatively safe status, and the potential development of multiple uses.

The essential oils of some *Matricaria* species have been examined, for example, the main constituents of the Chamomile (*M. recutita* L.) essential oil were bisabolol, bisabolol oxides, chamazulene, farnesene and spiroether (Matos *et al*., 1993; Pino *et al*., 2002; Sashidhara *et al*., 2006; Karami *et al*., 2009; Gosztola *et al*., 2010; Orav *et al*., 2010). The constituents of the essential oil from *M. decipiens* C. Koch were isomers of matricaria ester, 1,8-cineole, farnesene and camphor (Javidnia & Shafiee, 1999). The essential oil of *M. Suffruticosa* var. leptoloba (Bohlmann & Zdero, 1975), *M. inodora* L. (Holman & Sorensen, 1950) and *M. perforata* (Baer & Schultze, 1996) was dominated by various isomers of matricaria ester.

M. songarica is mainly distributed in mountains of western China and Central Asia. This plant has been collected both as an important edible invigorant and medicinal herb to cure several ailments such as inflammatory, dermal ulcer, and bleeding in folk traditional medicine of Yunnan Province, China since ancestral times.

The aim of this study was to investigate the chemical composition of the hydrodistilled essential oil from *M. songarica* grown in the Gaoligong Mountains, Yunnan Province, China by GC-MS, and to evaluate its antimicrobial activity against several food microorganisms.

MATERIALS AND METHODS

Plant material: The whole grasses of *M. songarica* were collected in Gongshan, Yunnan Province, China in July, 2009, and were authenticated by Prof. Gong Xun, Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (No. 721349) was deposited.

Isolation of the essential oil: The air-dried plant materials (500 g) of *M. songarica* were chopped and submitted to hydrodistillation for 6 h in a Clevenger-type apparatus. The oils were dried over hydrous sodium sulfate for 24 h, after filtration stored at 4°C in the sealed brown glass vials until tested.

GC–MS analysis: The GC-MS analyses were performed in a GC-MS (gas chromatograph 6890 coupled to a selective detector mass spectrometer 5975 inert, Agilent Technologies, Palo Alto, CA, USA). Compound separation was carried out using an HP-5MS capillary column (30 m×0.25 mm i.d., with 0.25 μm membrane thickness, Agilent

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Technology). Helium was used as a carrier gas at a flow rate of 1 mL/min. Mass spectra were acquired at the electron ionization (EI) mode with ionization energy of 70 eV. The front inlet was kept at 250°C in split-less mode and the mass transfer line temperature was at 280°C. The temperature program as below: the initial temperature was 40°C for 1 min and then programmed to 250°C with a 3°C/min heating ramp, then held at 250°C for 20 min. The Kovats indices (KI) of the sample components were calculated on the basis of a homologous series of n-alkanes C_8-C_{25} (Alfa Aesar). The compounds were identified by comparison with Nist 05 and Wiley 7.0 database. Some major components were identified by co-injection with standards compounds. Relative percentage of compounds was obtained by integrating the peak area of the spectrograms.

Antimicrobial activity: The *in vitro* antimicrobial activities of the essential oil were evaluated against panel, which included laboratory control strains obtained from the China Center for Type Culture Collection (CCTCC): two Gram-negative bacteria (*Pseudomonas aeruginosa* CCTCC AB93066 *& Escherichia coli* CCTCC AB91112), two Gram-positive bacteria (*Bacillus subtilis* CCTCC AB93174 *& Staphylococcus aureus* CCTCC AB91093), two yeasts (*Hansenula anomala* CCTCC AY92046 *& Saccharomy cescerevisiae* CCTCC AY92042) and four moulds (*Aspergillus niger* CCTCC AF91004, *Chaetomium globosum* CCTCC AF 200039, *Mucor racemosus* CCTCC AF 93209 *& Monascus anka* CCTCC AF93208). They were maintained on an agar slant at 4□. Bacterial strains were cultured on MHB (Muller-Hinton Broth) at 37□ for 24 h, yeasts were cultured on SDA (Sabouraud Dextrose Agar) at 28□ for 48 h and fungal strains were cultured on SDA at 28□ for 120 h before testing.

The agar diffusion method (Murray *et al.,* 1995) was employed to determine the growth inhibition caused by the essential oil from *M. songarica* against the above strains. Sterilized petri plates (12 cm diameter) were poured 15 mL of LB medium and then dried. 0.1 mL inoculum containing 10^{7-8} CFU/mL of microorganisms suspension was poured into plates, then spread uniformly and dried for 5 min. The essential oil was emulsified in the water with 2% (V/V) Tween 80 as surfactant to make a 10% (W/V) stock solution. The pieces of round Whatman No. 1 sterile filter paper of 6 mm diameter were dipped with 10 µL/disc of essential oil and put on the surface of plates. Positive or reference control were prepared using standard antimicrobial agents, tetracycline and streptomycin (10 µg/disc). Negative control contained only 2% (V/V) Tween 80. All plates were incubated for bacteria at 37°C for 24 h, incubated for yeasts at 28□ for 48 h and incubated for fungi at 28□ for 120 h. The zones of growth inhibition diameter against the tested microorganisms were measured to evaluate antimicrobial activity of essential oil.

The minimum inhibitory concentration (MIC) of the essential oils against the test microorganisms was evaluated by micro-dilution broth method (CLSI, 2003). A series of dilutions of essential oil were prepared in MHB or SDA at final concentrations ranging from 0.05 to 200 µg/mL. The inocula of microorganisms were prepared from 12 h cultures and suspensions were adjusted to 0.5 McFarland standard suspensions. The tubes were dispensed into 1 mL different concentrations of essential oil and 10 uL inoculum. The control tubes contained only MHB or SDA and inoculum suspension. The positive or reference control were prepared using gentamicin. The inoculated tubes of bacteria were incubated at 37□ for 24 h, yeasts at 28□ for 48 h and fungi at 28□ for 120 h. The MICs was calculated as no visible growth of tested microorganism appeared, which were expressed in ug/ml.

Statistical analysis: The tests were conducted in triplicate and data from experiments were analyzed by SPSS 14.0 and calculated as mean±SD.

RESULTS AND DISCUSSION

Chemical composition of the essential oil: Gaoligong Mountains, located at the linking point among China, Indian sub-continent, and China–Indian Peninsula, is rich in species of different medicinal plants grown in various ecological conditions. Investigation of antimicrobial properties of these plants has brought the opportunity of producing natural-based and environment friendly new source that could be replaced with the synthetic antimicrobials. To the best of our knowledge, no *M. songarica* phytochemical and pharmacological study has previously been reported.

The steam distillation of 500 g of the air-dried plant yielded 3.2 mL (0.64% v/w) oil. The oil with a distinct smell was light green in colour. The constituents of the essential oil were analyzed by GC–MS. Total 55 compounds representing 97.55% of the oil were identified and the results are listed in Table I. Among these, the amount of matricaria ester was 15.37%, the oxygenated sesquiterpenoid was 34. 45%, the sesquiterpene hydrocarbon was 21.03%, the oxygenated monoterpene was 10.97%, and the monoterpene hydrocarbon was lower (3.65%). The major constituents detected were: E-β-farnesene (10.58%), bisabolol oxide A (10.46%), α-bisabolol (9.33%), (Z,Z)-matricaria ester (8.28%), (E,E)-farnesol (8.26%), and (E,Z)-matricaria ester (4.05%).

The results indicate the essential oil from *M. songarica* 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* (Bohlmann & Zdero, 1975; Baer & Schultze, 1996; Javidnia & Shafiee, 1999; Gosztola *et al*., 2010; Orav *et al*., 2010).

Antimicrobial activity: Food spoiling and borne microorganisms play an important role in preservation of various food systems and responsible for their economic value loss. Different synthetic compounds, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT),

Table I: Chemical composition of essential oil of *M. songarica*

Peak no.	RI[a]	Components	%RA[b]	Identification methods[c]
1	939	α-Pinene	0.29	MS, RI
2	954	Camphene	0.23	MS, RI
3	977	Sabinene	0.41	MS, RI
4	1011	d-3-Carene	0.62	MS, RI
5	1019	α-Terpinene	0.12	MS, RI
6	1025	p-Cymene	0.64	MS, RI
7	1030	β- Phellandrene	0.22	MS, RI
8	1032	Limonene	0.32	MS, RI
9	1035	1,8-Cineole	0.46	MS, RI
10	1039	*Z*-β-Ocimene	0.13	MS, RI,Co
11	1050	E-β-Ocimene	0.42	MS, RI
12	1051	Benzeneacetaldehyde	0.66	MS, RI
13	1062	γ-Terpinene	0.25	MS, RI
14	1064	Artemisia ketone	1.54	MS, RI
15	1065	Z-Sabinene hydrate	1.09	MS, RI
16	1098	α-Linalool	0.15	MS, RI
17	1102	α-Thujone	0.14	MS, RI
18	1114	β-Thujone	0.54	MS, RI,Co
19	1139	E-Sabinol	3.14	MS, RI,Co
20	1144	Camphor	0.35	MS, RI
21	1168	Borneol	1.23	MS, RI
22	1179	4-Terpineol	0.45	MS, RI
23	1190	α-Terpineol	1.24	MS, RI,Co
24	1208	E-Piperitol	0.65	MS, RI,Co
26	1228	Nerol	1.08	MS, RI
27	1343	α-Cubebene	1.56	MS, RI
28	1354	α-Terpinyl acetate	1.64	MS, RI
29	1374	α-Isocomene	1.08	MS, RI
30	1390	β-Elemene	0.24	MS, RI
31	1399	α-Funebrene	0.35	MS, RI
32	1411	Isocaryophyllene	0.38	MS, RI,Co
33	1419	β-Caryophyllene	0.24	MS, RI
34	1459	E-β-farnesene	10.58	MS, RI,Co
35	1486	Germacrene D	1.36	MS, RI
36	1491	(Z,E)-α-Farnesene	1.22	MS, RI
37	1500	(Z,Z)-Matricaria ester	8.28	MS, Co,Co
38	1517	(E,Z)-Matricaria ester	4.05	MS, Co,Co
39	1522	δ-Cadinene	0.21	MS, RI
40	1525	β-Sesquiphellandrene	0.41	MS, RI
41	1527	(Z,E)-Matricaria ester	3.04	MS, RI
42	1532	Bicyclogermacrene	1.81	MS, RI
43	1561	E-Nerolidol	0.37	MS, RI
44	1578	Spathulenol	3.47	MS, RI
45	1581	Caryophyllene oxide	0.30	MS, RI
46	1654	Bisabolol oxide B	2.26	MS, RI
47	1672	Bisabolol oxide A	10.46	MS, RI,Co
48	1685	α-Bisabolol	9.33	MS, RI,Co
49	1723	(E,E)-Farnesol	8.26	MS, RI,Co
50	1735	Chamazulene	2.34	MS, RI
51	1772	Guaiazulene	2.67	MS, RI
52	1788	Octadecene	0.95	MS, RI
53	1807	Z-ene-yne-Dicyclo ether	3.08	MS, Co
54	1815	E-ene-yne-Dicyclo ether	1.03	MS, Co
55	2112	E-Phytol	0.21	MS, RI
		Total identified (%)	97.55	
		Matricaria ester	15.37	
		Monoterpene hydrocarbons	3.65	
		Monoterpenoids	10.97	
		Sesquiterpene hydrocarbons	21.03	
		Sesquiterpenoids	34.45	
		Others	12.08	

[a]Retention index relative to n-alkanes on HP-5 MS capillary column
[b]Relative area (peak area relative to the total peak area)
[c]RI is the retention index, MS = mass spectrum, Co = co-injection with authentic compound

have been widely used as antimicrobial agents for many years to inhibit the infestations of food microorganisms, and to increase the storage and marketing shelf lives of foods (Cook, 2005). Although synthetic preservatives have been proved very effective, these compounds may possess mutagenic activity to non-target organisms (Tripathi *et al*., 2007) and cause environmental pollution (Misra & Pavlostathis, 1997). In recent years, the widespread demand from consumer and government that antimicrobial materials without toxicity and chemical residues is increasing. Therefore, much effort has been made to search new antimicrobial compounds from natural sources. The applications of essential oils from the plant in food preservation can be effective and there is increasing interest in this domain (Izadi *et al*., 2010).

The *in vitro* antimicrobial activity of *M. Songarica* essential oil obtained by hydrodistillation against the tested microorganisms was assessed by the presence or absence of inhibition zones and minimum inhibitory concentration values. The results shown in Table II indicate that the oils have exhibited moderate to high antimicrobial activity against the test microorganisms. As shown in Table II, tetracycline exhibited highest antimicrobial effect against all employed microorganisms except two Gram-positive bacteria (*B. subtilis* & *S. aureus*), while *M. Songarica* essential oil showed higher antimicrobial activity against all test microorganisms than that of standard streptomycin. As shown in Table II, the essential oil exhibited remarkable inhibitory effect on the growth of all microorganisms, with MIC values ranged from 25 to 200 µg/mL. The negative control did not show antimicrobial effect against test microorganisms at the concentration used in this study. According to our results, Gram-positive bacteria were more susceptible than Gram-negative bacteria to the antimicrobial activity of essential oil, which is in accordance with some previous reports (Burt, 2004; Al-Bayati, 2008; Izadi *et al*., 2010).

The antimicrobial activity of the essential oil from *M. songarica* may be associated with its major components such as farnesene, bisabolol oxide, bisabolol, matricaria ester, and farnesol. In previous studies, the antimicrobial effect of these components has been confirmed. Maria-Rose *et al.* (2004) claimed that farnesene have enormous potential against food spoilage and foodborne pathogenic bacteria. Pauli (2006) reported that α-bisabolol from *M. chamomilla* may inhibit fungal growth via specific inhibition of ergosterol biosynthesis. Lu *et al.* (1998) reported the antibacterial activity of six matricaria esters against *Mycobacterium tuberculosis* and *Mycobacterium avium*, using a radiorespirometric bioassay. Togashi *et al.* (2008) reported the antibacterial activity of farnesol against *Staphylococcus aureus*. Moreover, other components such as pinene, sabinene, limonene, artemisia ketone, α-linalool, E-sabinol, borneol, α-terpineol, nerol, α-cubebene, α-terpinyl acetate, α-isocomene, bicyclogermacrene, spathulenol, chamazulene, guaiazulene may also contribute

Table II: Antimicrobial activity and minimum inhibitory concentrations of essential oil of *M. songarica* against the growth of microorganisms

Microorganism	Diameter of zones of inhibition			[C]MICs of Essential oil
	[A]Essential oil	[B]Standard		
		SM	TC	
Pseudomonas aeruginosa	21.0 ± 1.0	20.9 ± 0.7	21.5 ± 0.6	200
Escherichia coli	21.5 ± 1.1	21.4 ± 0.5	22.4 ± 0.9	100
Bacillus subtilis	23.7 ± 0.9	20.8 ± 0.5	23.3± 0.7	50
Staphylococcus aureus	23.8 ±0.9	20.7 ± 0.7	23.5 ± 0.8	50
Hansenula anomala	21.4 ±0..7	21.0± 0.8	23.2 ± 0.6	50
Saccharomy cescerevisiae	22.0 ± 0.5	20.6 ± 0.5	22.8 ± 0.4	50
Aspergillus niger	21.7 ± 0.7	21.0 ± 0.4	21.9 ± 0.6	25
Chaetomium globosum	22.1 ± 0.8	20.7 ± 0.7	22.6 ± 0.8	25
Mucor racemosus	21.6 ± 0.5	21.2 ± 0.6	21.9 ± 0.8	50
Monascus anka	22.3 ± 0.9	21.2 ± 0.5	22.6 ± 0.7	50

Diameter of inhibition zones (mm) including the diameter of disc (6 mm), values are given as mean ± SD of triplicate experiment

[A] Diameter of inhibition zones of essential oil (tested volume 1000 µg/disc)

[B] Standard antibiotics: SM, streptomycin; TC, tetracycline (tested volume 10 µg/disc)

[C] MIC, Minimum inhibitory concentration (values in µg/ml)

to antimicrobial activity of oil. In our opinion, it is also possible that the antimicrobial activity of oil is a synergistic effect of major constituents and minor constituents present in the essential oil.

CONCLUSION

The essential oils from plants caused great interest in consumer and researcher because of antimicrobial properties of essential oils and the possibility that these oils are used to replace chemical antimicrobial agent. To the best of our knowledge, chemical composition and antimicrobial activity of the essential oil from *M. songarica* is reported for the first time and the results obtained in this study are consistent with the traditional uses of *M. songarica* to some extent. The oil may be suggested as a valuable source in the foods as a natural antimicrobial agent. Further researches are needed to get more information on safety and toxicity of this oil.

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