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Full Length Research Paper

Antimicrobial activity and chemical composition of essential oils of chamomile from Neyshabur, Iran

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Matricaria chamomilla **L. or German chamomile is an annual plant of the composite family Asteraceae. In this study, *M. chamomilla* L. were collected from Neyshabur, Iran. Chemical constituent of essential oils of *M. chamomilla* L. were determined. Aerial parts (200 gr) were subjected to hydrodistillation in a Clevenger – type apparatus until there was no significant increase in the volume of the oil collected (2.5 h). The yield of the blue oil was 0.9% (w/w). The essential oil was analyzed by GC and GC/MS. Identification of the components was based on GC retention indices computer matching with Wiley GC-MS library, and by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Adams, 2004). 47 components were identified constituting more than 83.1% of the oil. p-Cymene-8-ol(0.7%), Azulene(0.4%), p-Cymene (1.1%), 1,8-Cineole (2.1%), Artemisia Alcohol (0.2%), β-Elemene (0.9%), cis-β-farnesene (0.9%), trans-β-farnesene (5.2%), Borneol (0.8%), γ-Cadinene (0.4%), Spathulenol (9.4%), γ-Eudesmol (1.5%), α-Bisabolol oxide B(7.0%), α-Bisabolol oxide A (21.5%), α-Bisabolol (5.0%), Chamazulene (4.2%) and Germacrene D (0.8%) were major components in *M. chamomilla* L. oil. The oil was tested against seven strains of bacteria (Gram-positive and Gram-negative). The oil showed mild to significant antimicrobial activity associated mainly with Gram-positive and Gram-Negative bacteria.**

Key words: *Matricaria chamomilla* L., essential oil, antimicrobial activity, α-Bisabolol oxide A, α-Bisabolol oxide B.

INTRODUCTION

During recent years, there has been a growing increase in substances exhibiting antimicrobial properties that are supplied to human and animal organisms as food components or as specific pharmaceutics (Azuma et al*.,* 1995). Plant essential oil and extracts are the sources of naturally occurring antioxidants. Essential oils exhibit some antimicrobial properties (Ozer et al., 2007*)*.

Matricaria chamomilla L. or German chamomile also spelled camomile is an annual plant of the composite family Asteraceae. It usually grows near populated areas all over Europe and temperate Asia. Because the seeds need open soil to survive, it often grows near roads, around landing and in cultivated fields as a weed (Plant database USDA, 2008; Integrated toxonomic information system).

M. chamomilla L. is an annual plant approximately 50 cm, with drawn up stem, oarswoman. The branched stem is erect and smooth and grows to a height of 15 to 60 cm. The long and narrow leaves are bipinnate or tripinnate. The flowers are borne in paniculate capitula. The white ray florets are furnished with a ligule, while the disc florets are yellow. The hollow receptacle is swollen and lacks scales. This property distinguished German Chamomile from, Corn chamomile (*Anthemis arvensis*), which has a receptacle with scales. The flowers have a strong, aromatic smell and bloom in early to mid summer medically. German chamomile is used to treat sore stomach, irritable bowel syndrome, and as a gentle sleep aid. It is also used as a mild laxative and is anti-inflammatory and bactericidal (Fatouma et al., 2011; Shivananda et al., 2007; Owlia, 2007). It can be taken as a herbal tea, which should be steeped for ten to fifteen minutes while covered to avoid evaporation of the volatile oils. The marc should be pressed because of the formation of a new active principle inside the cells, which can then be released by rupturing the cell walls, though this substance only forms very close to boiling point. For a sore stomach, some recommend taking a cup every

morning without food for two to three months. It is also used as a mouthwash against oral mucositis. It has acaricidal properties against certain mites, such as *Psoroptes cuniculi*. One of the active ingredients of the essential oil from German chamomile is the terpene bisabolol. Other active ingredients include farnesene, chamazulene, flavonoids (including apigenin, quercetin, patuletin and luteolin) and coumarin (McKay and Blumberg, 2006). Chamomile (*M. chamomilla*), a popular herb valued for centuries as a traditional medicine, has been used to treat various human ailments; however, its anticancer activity is unknown (Janmejai K, et al. 2007). Antifungal activity of *M*. *chamomilla* flower essential oil collected from Karaj, Iran, has been investigated against *Aspergillus niger* and identified as having 21 components in the plant oil (92.88%w/w), include bisabolol oxide A (2.19%) and chamazulene (2.18%) (Tolouee et al., 2010). Antimicrobial and antioxidant activities of essential oil and methanol extracted of *M. chamomilla* L. collected from Djibouti were investigated (Fatouma et al., 2011).

Chemical composition of volatile essential oil from a *chamomile* sample cultivated in Botanical Garden of School of pharmacy in Isfahan, Iran, was studied. The oil plant represented 86.21%w/w of total oil and 63 components were characterized. Bisabolol oxide A (25.01%) and bisabolol oxide B (9.43%) were the major constituents of the oil (Shams Ardakani MR et al., 2006). Inhibitory effect of essential oils against herpes simplex virus type 2 was investigated (Koch et al., 2008). In a clinical study chamomile solution or a 1% topical hydrocortisone ointment was used in the management of peristomal skin lesions in colostomy patients (Charousaei et al., 2011). Anti-allergic activity of German chamomile (*Matricaria recutita* L.) in mast cell mediated allergy model was studied (Chandrashekhar et al., 2011). Antihyperglycemic and antioxidative potential of *M. chamomilla* L. in streptozotocin-induced diabetic rats was investigated (Mustafa et al., 2008). Antiproliferative and apoptotic effects of chamomile extract in various human cancer cells were studied (Janmejai et al., 2007).

MATERIALS AND METHODS

Plant material

Essential oil of *M. chamomilla* collected from Neyshabur, Khorasan-Razavi Province, Iran, in 2010 was obtained by hydrodistillation of the aerial parts. Voucher specimens of the plant have been deposited in the herbarium.

Isolation of the essential oil

Aerial parts (200 gr) of *M. chamomilla* were subjected to hydrodistilation using a Clevenger-type apparatus for 2.5 h. After decanting, the obtained essential oil was dried over anhydrous sodium sulfate and, after filtration, stored in refrigerator at -4℃ until tested and analyzed.

Gas chromatography and GC-MS

GC analysis was performed on a shimadzu 15 A gas chromatography equipped with split/splitless injector (250℃) and a flame ionization detector (250℃). Nitrogen was used as carrier gas (1 ml/min) and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60℃ for 3 min and then heated to 220℃ with a 5℃ / min rate and kept constant at 250℃ for 5 min. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

GC-MS analysis was performed using a Hewlett – Packard 5973 with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µM). The column temperature was kept at 60℃ for three minutes and programmed to 220℃ at a rate of 5℃ / min and kept constant at 220℃ for five minutes. The flow rate of Helium as carrier gas (1 ml/min). MS were taken at 70 eV.

Identification of the constituents of each oil was made by comparison of their mass spectra and retention indices (RRI) with those given in the literature and those authentic samples (Adams, 2004). Relative percentages of components were obtained from the peak area percent reports of volatiles from GC/MS data in Table 1.

Antimicrobial assay of the oil

In vitro antibacterial assay of the oil carried out according to disc agar diffusion method (Jirovets et al., 1999; Ajai et al., 2004). Antibacterial activity of the oil was tested against Gram-positive bacterial strains such as *Bacillus cereus* (MTCC430), *Bacillus subtilis* (MTCC441), *Staphylococcus aureus* subsp. *aureus* (MTCC2940) and Gram-negative bacterial strains; *Klebsiella pneumonia* (MTCC109), *Escherichia coli* (MTCC443), *Proteus vulgaris* (MTCC426) and *Salmunella typhi* (MTCC733) were grown in nutrient broth for 24 h (pH 7.2 to 7.4) and were used as inoculums. The Mueller-Hinton agar medium were poured into the plates to uniform depth of mm and allowed to solidify. Then the microbial suspentions were streaked over the surface of media using a sterile cotton swab to ensure the confluent growth of the organism. Aliquots of 10 µl of the oil at 1:2 dilutions in dimethyl sulfoxide (DMSO) were impregnated on Whatman No. 1 filter paper discs of 6 mm diameter. These discs were aseptically applied to the surface of the agar plates at well-spaced intervals. The plates were incubated at 37℃ for 24 h and observed inhibition zones including the diameter of the discs were measured. Control discs impregnated with 10 µl of the solvent DMSO and streptomycin (10 µl /disc), reference for bacteria were used alongside the test discs in each experiment. The results are presented in Table 2.

RESULTS AND DISCUSSION

The volatile oil obtained in 0.90% w/w yield from 200 gr air dried aerial parts of *M. chamomilla* was blue color. 47 components identified (out of 57 existing components) in oil. Among them monoterpenes were 3.7%, oxygenated monoterpene were 4.3%, sesquiterpenes were 11.6%, oxygenated sesquiterpenes were 58.8% and other components were 4.6%. p-Cymene-8-ol (0.7%), Azulene(0.4%), p-Cymene (1.1%), 1,8 - Cineole(2.1%), Artemisia Alcohol (0.2%), β-Elemene (0.9%),

Table 1. Chemical composition (%) of essential oil of aerial parts *Matricaria chamomilla L.*

Compounds	Aerial parts (%)	RRI
α-Pinene	0.1	958
para-Cymene	1.1	1024
(Z)-β-ocimene	0.2	1033
1, 8-cineole	2.1	1034
(E)-β-ocimene	0.1	1042
Limonene	0.2	1034
γ-Terpinene	0.3	1052
Cis-sabinene hydrate	0.2	1062
Artemisia alcohol	0.2	1062
n-Nonanal	0.1	1075
Camphor	0.1	1099
Linalool	0.1	1100
Cis-Chrysanthenol	0.1	1114
Borneol	0.8	1117
4-Terpineol	0.1	1171
Para-Cymene-8-ol	0.7	1182
Nonanoic acid	0.3	1201
Azulene	0.4	1218
Daucene	0.5	1253
Cis-β-farnesene	0.9	1321
Trans-β-farnesene	5.2	1331
δ-Elemene	0.1	1331
α-Muurolene	0.8	1355
Cis-α-Bisabolene	0.3	1360
β-Bisabolene	0.2	1364
γ-Cadinene	0.4	1366
α-Copaene	0.2	1368
δ-Cadinene	0.2	1372
Trans-γ-Bisabolene	0.1	1376
β-Elemene	0.9	1386
α-Cadinene	0.9	1386
β-Caryophyllene	0.3	1410
Spathulenol	9.4	1419
γ-Eudesmol	1.5	1462
β-Selinene	0.5	1475
α-Bisabolol oxide B	7.0	1476
β-Bisabolol	0.1	1482
γ-Elemene	0.7	1431
Germacrene-D	0.8	1471
α-Bisabolene oxide A	10.0	1493
α-Bisabolol	5.0	1499
Chamazulene	4.2	1524
α-Bisabolol oxide A	21.5	1557
Caryophyllene oxide	0.7	1573
β-Bisabolenal	0.8	1578
Juniperol	0.9	1591
α-Bisabolol acetate	1.8	1602

Table 1. Contd.

Grouped components	
Number of constituents	57
Number of identified constituents	47
Percentage identified	82
Percentage of Monoterpene hydrocarbons	3.7
Percentage of oxygen-containing monoterpenes	4.3
Percentage of sesquiterpene hydrocarbons	11.6
Percentage of oxygen-containing sesquiterpenes	58.8
Percentage of others	4.6
Percentage of total	83.1

Table 2. Antibacterial activity of the aerial parts oil of *Matricaria chamomilla L.*

Tested bacteria	**MTCC No.**	**Zone of inhibition (mm)**	
		Oil in DMSO (1:2)	**Streptomycin 1 mg/ml**
Gram-positive bacteria			
Bacillus cereus	430	11.5	7.5
Bacillus subtillis	441	8	11
Staphylococcus aureus subsp. Aureus	2940	13.5	12.5
Gram-negative bacteria			
Escherichia coli	443	8	13
Klebsiella pneumoniae	109	9	9.5
Proteus vulgaris	426	11.5	10
Salmunella typhi	733	8.5	9

cis-β-farnesene (0.9%), trans-β-farnesene (5.2%), Borneol (0.8%), γ-Cadinene (0.4%), Spathulenol (9.4%), γ-Eudesmol(1.5%), α-Bisabolol oxide B (7.0%), α-Bisabolol oxide A (21.5%), α-Bisabolol (5.0%), Chamazulene (4.2%), and Germacrene D(0.8%) were some major components in *M. chamomilla* L. oil. The oil in DMSO (1:2 dilutions) showed 153% inhibition against *B. cereus*, 73% inhibition against *B. subtilis* and 108% inhibition against *S. aureus* subsp. *aureus* as compared to the standard, streptomycin at 10 µl/disc. The oil showed comparable activity against Gram-negative bacteria: 95% against *K. pneumonia*, 62% against *E. coli*, 115% against *P. vulgaris* and 94% against *S. typhi* (Table 2).

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