Short Communication

Eugenol-rich essential oil of *Anthemis mazandranica* and its antibacterial activities

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The hydro-distilled volatile oil obtained from aerial parts of *Anthemis mazandranica* was analyzed by gas chromatography and mass spectrometry (GC-MS). Seventeen compounds were identified, representing 93.35% of the total oil composition, with eugenol (35.5%) being the main component. Oil antimicrobial activity was carried out using the disk diffusion and minimal inhibitory concentration (MIC). The best antibacterial activity was observed against *Salmonella Typhi* with $ZI = 19 \pm 0.5$ mm and MIC value of 32 µg/mL.

Key words: *Anthemis mazandranica*, essential oil, antimicrobial, eugenol.

INTRODUCTION

The genus *Anthemis*, comprises 130 species widespread in the Mediterranean, South West Asia and South Africa (Kudryashev, 1932). Present in Iran are 39 species growing wild, among which 15 are endemic (Mozaffarian, 1996). From the Roman times up to now, *Anthemis* taxa have been commonly used as folk remedies, insecticides and dyes (Niko et al., 2009). Water-distilled essential oils from the leaves and flowers of *A. altissima* (L.) Var. *Altissima*. was analyzed by GC-MS. β -Thujone (33.7 and 19.7%, respectively) was found as the major constituent in the leaf and flower oil (Rustaiyan et al., 2004). Over the last two decades, *Anthemis* volatile compounds have received more attention (Vuckovic et al., 2006; Williams et al., 2001; Vajs et al., 1999).

MATERIALS AND METHODS

Plant material and isolation procedure

The aerial parts of *A. mazandranica* growing wild in Shiraz (Provincial capital of Fars) was collected at the flowering stage in May, 2011. Their identities were confirmed by Dr. Valiollah

Mozaffarian and a voucher specimen (no. VS-21-13) was deposited at the Herbarium of Science and Research Branch, Islamic Azad University (Tehran, Iran).

Hydrodistillation

The air-dried aerial parts (leaves, petals and stems) (100 g) were dried, powdered and the volatile fraction was isolated by hydrodistillation for 3 h using a Clevenger-type apparatus. The essential oil had a bright yellow color and yielded 0.59% w/w.

GC and GC/MS analysis

GC analysis of the oil was performed using a Shimadzu 15 A gas chromatograph equipped with flame ionization detector (FID) and a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Temperature program: 60°C (3 min), 60 to 220°C at 5°C/min, 220°C (5 min); injector and detector temperatures, 260°C; the carrier gas was N₂ (1 ml/min). The sample was injected in split-splitless mode, using a split ratio of 1:50. The percentages of each component were reported as raw percentages without standardization. GC-MS analysis was carried out on a Hewlett-Packard 6890/5973 using an HP-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was as above,

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Table 1. Chemical composition of leaves and aerial parts oils of *A. mazandranica*.

Compound	RI ^a	Theoretical value	Aerial part (%)
Octane	800	800	2.28
Decane	1000	1000	3.59
α -Phellandrene	1003	1002	3.41
p-Cymene	1025	1089	2.99
limonene	1029	1024	3.45
β -Phellandrene	1030	1025	0.61
1,8-Cineole	1034	1026	4.04
Myrcenyl acetate	1327	1312	1.88
Cyclohexasiloxane, dodecamethyl	1340	1330	18.64
Eugenol	1359	1356	35.55
E-jasmone	1402	1390	1.87
β -Caryophyllene	1419	1417	6.94
Trans- α -Bergamotene	1438	1432	2.52
γ -Eudesmol	1621	1630	3.17
α -Cadinol	1654	1052	0.72
Monoterpene hydrocarbons			10.46
Oxygenated monoterpene			59.11
Sesquiterpene hydrocarbons			9.47
Oxygenated sesquiterpene			3.89
Total			91.66
Yield, w/w%			0.59

^a Kovat's retention index, Tr: trace (< 0.05%).

interface temperature, 260°C; mass range was 40 to 300 amu; scan time, 1 s. Retention indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C6 to C25) with linear interpolation. Identification of the oil components was done by comparison of their mass spectra with Wiley 275 GC-MS library, by comparing them with those reported in the literature and confirmed by comparison of its retention index either with those of authentic compounds or with data in the literature (Jenning and Shibamoto, 1980; Adams, 1995).

Antimicrobial activity

All test microorganisms were obtained from the Persian type culture collection (PTCC), Tehran, Iran and were as follows: *Bacillus pumilus* (PTCC 1319), *Escherichia coli* (PTCC 1533), *Kocuria varians* (PTCC 1484), *Pseudomonas aeruginosa* (PTCC 1310), *Salmonella Typhi* (PTCC 1609), and *Listeria monocytogenes* (PTCC 1298).

Assessment of antimicrobial activity

The antibacterial activity of the *A. mazandranica* essential oil was screened against Gram-positive and Gram-negative bacteria, with two methods:

Disc diffusion assay

Antimicrobial tests were carried out by the disc diffusion method reported by Murray and his co-worker in 1999 (Wayne, 2006). The

dried *A. mazandranica* essential oil was dissolved in Dimethyl sulfoxide (DMSO) to a final concentration of 30 mg/ml and filtered through 0.45 μ m Millipore filters, using 100 μ l of suspension containing 108 CFU/ml of bacteria and 104 spore/ml of fungi spread on the nutrient agar (NA) and potato dextrose agar (PD) mediums, respectively. The discs (6 mm in diameter) impregnated with 10 μ l of the essential oil solution (300 μ g/disc) and DMSO (as negative control) were placed on the inoculated agar. The inoculated plates were incubated for 24 h at 37°C for bacterial strains and 48 and 72 h at 30°C for mould isolates, respectively. Gentamicin (10 μ g/disc) and ampicillin (5 μ g/disc) were used as positive controls for bacteria. The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was repeated twice.

MIC agar dilution assay

The lowest concentration of the compounds that prevented visible growth was considered as the minimal inhibitory concentration (MIC). MIC value of the plant essential oil against standard bacterial strains was evaluated based on the agar dilution method. Appropriate amounts of the *A. mazandranica* oil were added aseptically to sterile molten Sabouraud dextrose agar (SDA) medium added with Tween 20 (0.5%, v/v) to produce the concentration range of 8 to 500 μ g/ml. The resulting SDA agar solutions were immediately mixed and poured into Petri plates. The plates were spot inoculated with 5 μ l (104 spore/ml) of each fungus isolate. At the end of incubation period, the plates were evaluated for the presence or absence of growth. Ampicillin and tetracycline were used as references for gram-positive and negative bacteria, respectively. The MIC was defined as the lowest concentration of the oil needed to inhibit the growth of microorganisms. Each test was repeated at least twice.

Table 2. Antimicrobial activity of the aerial parts essential oil of *A. mazandranica*.

Microorganism	MIC ($\mu\text{g/ml}$) of <i>A. mazandranica</i>	MIC ($\mu\text{g/ml}$) of reference ^a	ZI (mm) of <i>A. mazandranica</i> ^b	ZI (mm) of reference ^c
<i>Bacillus pumilus</i>	128	64	11.5 $\pm$ 0.5	16.3 $\pm$ 0.5
<i>Escherichia coli</i>	128	16	10 $\pm$ 0.1	16 $\pm$ 0.0
<i>Kocuria varians</i>	64	32	13.5 $\pm$ 0.5	17.6 $\pm$ 0.5
<i>Listeria monocytogenes</i>	512	16	6.5 $\pm$ 0.5	14.3 $\pm$ 0.5
<i>Pseudomonas aeruginosa</i>	64	8	14.5 $\pm$ 0.5	16.3 $\pm$ 0.1
<i>Salmonella Typhi</i>	32	32	19.5 $\pm$ 0.5	21.3 $\pm$ 0.5

^aAmpicillin, tetracycline were used as references for Gram-positive, Gram-negative bacteria, respectively. ^bZI: Zone of Inhibition. Including the diameter of the filter paper disc (6 mm); mean value of four experiments $\pm$ SD. Campicillin (5 $\mu\text{g/disc}$), gentamicin (10 $\mu\text{g/disc}$) were used as references for Gram-positive and negative bacteria, respectively.

RESULTS AND DISCUSSION

The oil of the aerial part contained 19 compounds with a yield of 0.59% (w/w), representing 93.35% of the total oil composition (Table 1), where main component was eugenol (35.5%). In particular, Oxygenated monoterpenes (59.11%) were the most abundant group of compounds. In this study, the antimicrobial activities of the aerial parts essential oil of *A. mazandranica* were investigated against six bacterial standard strains in laboratory situation (*in vitro*). Antimicrobial activity (inhibition zone and MIC) of the oil against standard microorganisms is shown in Table 2. The best antibacterial activity was observed against *S. Typhi* (ZI and MIC value 19 $\pm$ 0.5 mm and 32 $\mu\text{g/ml}$, respectively). From a medical point of view, the antimicrobial activity of *A. mazandranica* oil against *S. Typhi* is particularly interesting because of the role of this microorganism as a pathogen agent responsible for severe typhoid fever infection.

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