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Research Article

Evaluation of Antimicrobial Activity of Essential Oils from Different Parts of *Anthemis odontostephana* Boiss. Var. *odontostephana*

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ABSTRACT

In this research essential oils isolated from different parts including flowers, leaves, stems and roots of *Anthemis odontostephana* Boiss. var. *odontostephana* by hydrodistillation in a clevenger extractor and chemical composition were identified by GC/FID and GC/MS. *Anthemis odontostephana* Boiss. var. *odontostephana* belonging to Asteraceae family is one of the *Anthemis* species that distributed in central and south of Iran. The aim of this study was chemical composition and evaluate the antimicrobial activity of essential oils obtained from different parts of *Anthemis odontostephana* Boiss. var. *odontostephana*. Borneol was the major chemical compound in flowers, leaves and stems. In roots essential oil Pentadecanoic acid was the major compound. Different essential oils were also tested for antimicrobial activity against 9 microorganism, by using broth microdilution method and results show that, the strong inhibitory activity with MIC values ranging 32-64 µg/ml against the bacteria and fungal strains. Hence, the essential oils obtained from *A. odontostephana* Boiss. var. *odontostephana* could be good candidate for used at further investigation.

Keywords: *Anthemis odontostephana* Boiss. var.*odontostephana*, Antimicrobial activity, Broth microdilution method, Essential oil

INTRODUCTION

The genus *Anthemi*s is belonging to the family Asteraceae and comprises about 130 species in the world[1] and this is one of the best phytochemically investigated genera of the family Asteraceae[2]. Thirty-nine species of *Anthemis* could be found in flora of Iran[3]. The species of the *Anthemis* genus are widely used in the pharmaceutical, cosmetics and food industry[4]. Several *Anthemis* species are used in the Iranian traditional pharmaceutical as medicinal plants[5]. The flowers of the genus have well-documented use as antiseptic and healing herbs[6]. The main components of *Anthemis* species, including natural flavonoids and essential oils. Natural distinct flavonoids such as flavonol glycosides obtain at the leaf of *Anthemis* species, whereas the leaf flavonoids of other plants generally are flavone *O*-glycosides[7]. Several investigations have been performed showing that sesquiterpene lactones isolated from some *Anthemis* genus are used chemotaxonomic markers[8]. The essential oil composition of some *Anthemis* species have been reported in the literature. Previous result shows that *Anthemis* oils are generally dominated by oxygenated monoterpenes and sesquiterpene[9-14].

The essential oil from the flower of *A.odontostephana* Boiss.var.*odontostephana* has been studied and Spathulenol (24.1 %), Hexadecanoic acid (12.1 %), Germacrene D (6.9 %), 1,8-cineole (5.6 %) were found to be the major constituents of the essential oil[15].

Recently, there has been considerable interest in essential oils from aromatic plants with antimicrobial activity for controlling pathogens[16]. Antimicrobial activity of essential oil, crude extract and fractions of some *Anthemis* species studied and result show that some *Anthemis* species very effective against Gram-positive bacteria and negative bacteria and candida[4,13,17,18]. Antioxidants are a grouped of substance when present at low concentration compared to oxide substrates significantly inhibit or delay oxidative processes, while being oxidized themselves[19]. It has been established that oxidative stress is one of the major causative factor in induction of many chronic and degenerative diseases, including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer and other[20]. Although antioxidant activity of some *Anthemis* species was reported in the previous study[21,22] but after a thorough review of available literature on comparative study of the composition, antimicrobial activity of the flower, leaf, stem and root essential oil of *A. odontostephana* Boiss. var. *odontostephana* not been found.

Anthemis odontostephana Boiss. var. *odontostephana* is one of the wild species of *Anthemis* that distributed in central and south of Iran. The aerial parts of mentioned plant are 20-30 cm long with a pleasantly fragrant. In folk medicine, it has been used as an antispasmodic, anti migraine and hypolipidemic agent.

Present study for the first time evaluated of antimicrobial activity all part of the essential oils of *A. odontostephana* Boiss. var.*odontostephana*.

MATERIALS AND METHODS

Plant Material and Essential oil Preparation

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A. odontostephana Boiss. var. *odontostephana* was collected in May 2014 from Kuhenjan, Fars province south of Iran, during the flowering stage. The plant was air dried in the shade and identified by Dr. Farjam Medicinal Plants Research Institute Herbarium (Voucher number: IAF-32) Islamic Azad University, Firoozabad, Iran. Different parts of the whole plant separated and each part including flower 70 g, leaf 41 g, stem 75 g and root 56 g used for the obtained essential oil from hydrodistillation by Clevenger-type glass apparatus for 3 hours. Obtained oils, dried by sodium sulfate and kept in 4ºC for further steps.

Table 1. Chemical Composition of Essential Oils from Different Parts of *A.odontostephana* Boiss. var.*odontostephana*

Compound	KI[c]	KI[r]	Oil content (%)				Identification
			Flower	Leaf	Stem	Root	methods
α-Pinene	935	939	0.3	----	-----	-----	MS-KI
Camphene	951	953	3.6	0.7	1.1	0.5	MS-KI
Myrcene	993	990	1.4	3.8	3.0	1.1	MS-KI
Mesitylene	997	996	1.1	0.6	0.9	0.6	MS-KI
Trimethyl benzene 1,2,4	1025	1025	1.2	0.4	0.6	----	MS-KI
o-Cymene	1026	1026	----	----	----	1.3	MS-KI
γ-Terpinene	1066	1059	----	----	----	0.2	MS-KI
Filifolone	1106	1103	3.2	0.4	1.2	0.6	MS-KI
Isophorone	1123	1121	----	----	----	0.2	MS-KI
Chrysanthenone	1128	1128	9.7	3.7	5.3	1.4	MS-KI
Terpineol-1	1138	1133	----	0.2	----	----	MS-KI
cis-Verbenol	1143	1140	----	0.2	----	----	MS-KI
trans-Verbenol	1144	1144	----	----	----	1.2	MS-KI
Camphor	1148	1146	2.3	1.9	2.3	----	MS-KI
Pinocarvone	1162	1164	----	----	----	0.9	MS-KI
Borneol	1170	1169	31.3	19.2	27.0	8.2	MS-KI
Terpinen-4-ol	1183	1177	0.4	-----	----	----	MS-KI
trans-Carveol	1219	1216	0.3	0.7	0.6	0.3	MS-KI
Isobornyl formate	1232	1233	0.6	0.2	0.3	----	MS-KI
Carvacrol methyl ether	1238	1244	----	----	----	0.2	MS-KI
Thymol	1292	1290	----	3.5	2.9	2.9	MS-KI
(-)-Bornyl acetate	1293	1291	13.9	6.6	7.9	4.0	MS-KI
Carvacrol	1302	1299	----	0.4	0.4	0.5	MS-KI
Eugenol	1359	1359	----	0.3	0.3	0.1	MS-KI
Isobornyl propanoate	1379	1381	0.7	0.3	0.4	0.4	MS-KI
Tetradecene-1	1394	1389	----	0.2	0.2	0.2	MS-KI
β-Elemene	1396	1390	1.4	----	----	----	MS-KI
β-Caryophyllene	1420	1419	1.7	1.0	1.8	1.8	MS-KI
(E)-*β*-Farnesene	1459	1458	----	1.3	0.8	1.8	MS-KI
Myristicin	1514	1518	9.8	13.3	11.2	8.7	MS-KI
δ-Cadinene	1525	1523	----	0.3	----	0.3	MS-KI
trans -Nerolidol	1558	1563	1.0	7.1	6.1	10.9	MS-KI
Dodecanoic acid	1567	1566	1.2	1.3	1.2	1.1	MS-KI
Spathulenol	1575	1575	----	----	----	0.3	MS-KI
Caryophyllene oxide	1590	1583	1.4	1.0	0.9	0.7	MS-KI
Ledol	1610	1602	----	----	----	1.2	MS-KI
Tetradecanal	1611	1612	0.9	0.5	1.1	----	MS-KI
epi-Cedrol	1615	1619	0.4	0.9	0.8	----	MS-KI
γ-Eudesmol	1637	1632	----	1.3	1.1	2.1	MS-KI
Caryophylla-4(12),8(13)-dien-5-*β*-ol	1643	1640	0.8	0.6	0.6	0.3	MS-KI
Agarospirol	1646	1648	----	----	---	1.3	MS-KI
β-Eudesmol	1657	1651	----	1.2	0.7	5.1	MS-KI
α-Cadinol	1659	1654	1.0	1.7	1.4	1.3	MS-KI
β-Bisabolol	1676	1675	----	0.4	----	----	MS-KI
Elemol acetate	1677	1680	----	----	----	0.5	MS-KI
(2Z,6Z)-Farnesol	1696	1698	0.3	0.2	----	0.2	MS-KI
Benzyl benzoate	1758	1760	0.5	0.3	----	----	MS-KI
Myristic acid	1761	1768	----	----	----	0.2	MS-KI
γ-Eudesmol acetate	1781	1784	----	0.4	0.3	0.5	MS-KI
(2Z,6E)-Farnesyl acetate	1817	1822	----	----	----	0.1	MS-KI

Phytone	1847	1849	----	0.5	0.4	0.3	MS-KI
n-Hexadecanol	1881	1875	1.0	0.6	0.6	0.8	MS-KI
Pentadecanoic acid	1888	1887	0.4	9.6	6.0	15.1	MS-KI
Nonadecane	1889	1900	----	1.4	----	1.3	MS-KI
Methyl hexadecanoate	1924	1921	----	0.4	----	1.0	MS-KI
Hexadecanoic acid	1957	1960	0.8	----	----	----	MS-KI
Geranyl linalool(z,z)	1962	1961	0.3	3.6	3.4	11.3	MS-KI
Ethyl hexadecanoate	1995	1993	----	0.5	0.3	0.3	MS-KI
Ethyl linoleate	2161	2159	----	1.0	----	0.2	MS-KI
Not identified			2.7	3.6	4.0	1.5	
Unknown			3.6	1.7	2.5	4.3	
Total identified			93.7	94.7	93.5	94.2	

KI^c Kovats retention indices relative to C_8-C_{30} *n*-alkane on HP-5MS capillary column

KI^r Kovats retention indices base on (Adam, 2007)

Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS)

Essential oils were analyzed by GC and GC/MS. GC analysis was carried out using a Shimadzu 15A. An DB-5 capillary column (30m × 0.25mm i. d., film thickness 0.25 µm) was used with nitrogen as the carrier gas at a flow rate of (1ml/min). The oven temperature was kept at 60°C for 10 min and increased up to 250°C at a rate of 5°C/min and then kept constant at 250°C for10 min. The flame ionization detection (FID) detector temperature were 300°C and Injector temperatures were set at 280ºC. Diluted samples (1:5 V/V, in n-hexane) and 1 µl were injected manually at 250ºC, in the split/splitless mode. GC-MS analysis was the analyses of the volatile compounds were carried out on a Hewlet Packard 6890/5973 with a HP-5 MS capillary column (phenyl methyl siloxan, 30 m × 0.25 mm i.d.), and helium (He) was used as carrier gas with flow rate of (1ml/min). The mass spectrometer was the EI mode (70 ev). Mass rang was 40-600 *m/z.*

Identification Procedure

Identification and quantification of compound via GC/MS was based on a comparison of their MS spectra with Willey (nl7) and Adams library spectra, as well as with those reported in literatures. Further confirmation was done by referring to Kovats retention indices data calculated from series of normal alkane (C_8-C_{30})[23,24].

Microbial Strains

Antimicrobial activity all parts essential oils of *A. odontostephana* Boiss. var. *odontostephana* was investigated against three Gram-positive bacteria, including *Staphylococcus aureus* (PTCC 1431), *Staphylococcus epidermidis* (PTCC 1435), *Corynebacterium glutamicum* (PTCC 1532) and three Gram-negative bacteria viz., *Escherichia coli* (PTCC 1396), *Escherichia coli* (PTCC 1399), *Klebsiella bacteria* (PTCC 1053) and three fungi viz., *Aspergillus niger* (PTCC 5154), *Fusarium* solani species complex (PTCC 5284), *Alternaria alternata* (PTCC 5224). All microorganisms were obtained from the Persian type culture collection (PTCC), Tehran, Iran. All used strains in this study were human pathogenic.

Determination of Minimum Inhibitory Concentration (MIC)

Antimicrobial activity of all essential oils obtained from *A.odontostephana* Boiss. var.*odontostephana* was performed by determining the minimal inhibitory concentration (MIC). The lowest concentration of all the essential oils that inhibited visible growth was considered to be the MIC values. For bacterial and fungi strains the MIC of essential oils were tested in Muller Hinton broth by broth microdilution method[25-27] . The broth microdilution method, used for the bacterial strains and fungal, a range of concentrations of two-fold (from 4-512 µg/ml) in a 96-well plate. Each assay in this experiment was repeated twice. Bacterial strains were cultured overnight at 37°C in Müller-Hinton broth and fungi were cultured overnight at 30°C in sabouraud dextrose agar (SDA). The essential oils were dissolved in 5% DMSO to obtain 1000µg/ml stock solution. MCFarlands standard turbidity scale number 0.5 was prepared to give turbid solution. The control tube contained only organisms and not the sample. The inoculated plates were incubated for 24 h at 37°C for bacterial strains and 48 h at 30°C for fungal strain. And mold isolates, respectively. Antibiotics with positive responses were used as controls for the plates. Ampicillin, Gentamicin served as positive controls on Gram-positive and Gram-negative bacteria. Fluconazole served as a positive control on all fungal strains.

RESULTS AND DISCUSSION

Chemical composition of the essential oils

The yield extraction different essential oils including flower, leaf, stem and root were 0.7%, 0.5%, 0.7%, 0.2% (V/W), respectively. Table 1 represents the chemical composition essential oil all parts of *A.odontostephana* Boiss. var.*odontostephana.* (Table 1). The significance of bold in compound Borneol, (-)-Bornyl acetate, Myristicin, *trans* –Nerolidol, Chrysanthenone, Pentadecanoic acid, *β*-Eudesmol, Geranyl linalool (z,z) are to be noted these compound as the most abundant fractions. According to table 1 all identified compounds has been quantified by using peak areas and expressed as a relative percentage of the total compound. Totally thirty, forty three, forty six and forty eight compounds were identified in flower, leaf, stem and root, respectively. Major chemical compounds for the flower sample were Borneol (31.3%), (-)-Bornyl acetate (13.9%) followed by Myristicin (9.8%), Chrysanthenone (9.7%). Major constituents were as Borneol (19.2%), Myristicin (13.3%) followed by Pentadecanoic acid (9.6%), *trans* –

Table 2. Phytochemical Classification Compounds of Essential Oils from Different Parts of *A.odontostephana* Boiss. var.*odontostephana*

Grouped components	Flower	Leaf	Stem	Root
Monoterpenes hydrocarbons	5.3	4.4	4.1	3.0
Oxygenated monoterpenes	59.6	37.1	46.5	19.4
Sesquiterpene hydrocarbons	3.1	3.4	2.5	3.9
Sesquiterpenes oxygenated	5.3	18.6	15.5	35.5
Phenyl propanoid	9.8	13.6	11.5	9.7
Aliphatic hydrocarbons	4.1	1.7	3.0	1.9
Alkane hydrocarbons	----	1.38	----	1.4
Fatty acid	2.4	10.8	7.2	16.4
Ester	1.2	2.5	0.7	2.4
Others	2.9	1.2	2.4	0.6
	93.7%	94.7%	93.5%	94.2%

Nerolidol (7.1%) as well as (-)-Bornyl acetate (6.6%) for the leaf sample, Borneol(27.0%), Myristicin(11.2%) followed by (-)-Bornyl acetate(7.9%), as well as *trans* –Nerolidol(6.1%), Pentadecanoic acid(6.0%) and Chrysanthenone(5.3%) for stem sample and Pentadecanoic acid (15.1%), Geranyl linalool(z,z) (11.3%), *trans* –Nerolidol (10.9%) followed by Myristicn (8.7%), Borneol (8.2%) as well as *β*-Eudesmol (5.1%) for the root sample. Main ingredients for all samples are presented at the level of more than 5%. According to present findings Borneol is the major compound in flower, leaf and stem samples (31.3%), (19.2%) and (27.0%) but in root sample Pentadecanoic acid is the major compound (15.1%). Borneol is the oxygenated monoterpene and antithrombotic and antimicrobial effect of this compound have been reported in the previous study[28,29]. Previous investigation performed in flower essential oil of *A.odontostephana* Boiss. var.*odontostephana* show that Spathulenol is the major compound with (24.1%). Also in this research Hexadecanoic acid (12.1%), Germacrene D (6.9%), 1, 8-cineole (5.6%), 6-methyl-5-hepten-2-one (5.4%), *β*-Caryophyllene (4.8%) and Camphor (4.4%) were the all constituent compounds[15]. So the chemical composition from flower of the present study is different to previous study. It is necessary to point out that chemical compound any plant essential oil can greatly depend upon geographical region, the age of the plant, local climatic, seasonal and experimental condition[30,31]. Genetic differences are also responsible for the changes of chemical compounds[32]. Table 2 involved contents monoterpene hydrocarbones, oxygenated monoterpene, sesquitrepene hydrocarbones, oxygenated sesquiterpene as well as Phenyl propanoid and Fatty acid (Table 2). Accordingly, flower sample enriched in oxygenated monoterpene (59.6%). In addition to flower, the highest amount of oxygenated monoterpene compounds exist in leaf and stem samples (37.1%), (46.5%), respectively but in root sample oxygenated sesquiterpene compounds have a higher amount than the other (35.5%) and the oxygenated monoterpene is the second most abundant class of the root oil. The percentage of monoterpene hydrocarbon and sesquiterpene hydrocarbones involved lowest amount in the all samples. The highest amount of phenyl propanoid compounds exist in leaf sample (13.6%). Also the maximum amount of fatty acid exist in root sample (16.4%). Overall, findings on the profile of dominant terpene in essential oils of some *Anthemis* species are partly in line with some previous reports[2,10,32], but difference to those of others[14].

Antimicrobial activity

The minimum inhibitory concentration obtained in the broth microdilution assay (MIC) of the essential oils of *A. odontostephana* Boiss. var. *odontostephana* against 6 bacteria and 3 fungi are shown in (Table 3). The result showed all oils have significant antimicrobial against all tested bacteria and fungal strains in ranges 32-256 µg/ml than compared with positive controls. Essential oils complex mixture which constitutes mainly monoterpenes and sesquiterpenes. These constituents have been shown antimicrobial activity[14]. The action mechanism of terpenes

Table 3. Antibacterial and Antifungal Activity of Essential Oils from Different Parts of *A.odontostephana* Boiss. var.*odontostephana*

Test microorganisms		Essential oil				Antibiotics		
		Flower	Leaf	Stem	Root	Amp	Gen	Flu
Gram-negative bacteria		MIC (µg/ml)				MIC (µg/ml)		
Escherichia coli	PTCC 1396	32	64	128	64	8	16	8
Escherichia coli	PTCC 1399	128	64	64	128	8	16	8
Klebsiella bacteria	PTCC 1053	32	32	64	32	4	16	8
Gram-positive bacteria								
Staphylococcus aureus	PTCC1431	64	32	128	32	16	8	16
Staphylococcus epidermidis	PTCC 1435	128	32	128	64	8	16	4
Corynebacterium glutamicum	PTCC 1532	64	128	64	32	16	8	16
Fungi								
Aspergillus niger	PTCC 5154	32	32	64	32	8	8	16
Fusarium solani species complex (FSSC)	PTCC 5284	64	32	64	64	4	8	4
Alternaria alternata	PTCC 5224	32	64	32	64	8	8	4

MIC: Minimum Inhibitory Concentration (range of concentration: 4-512 µg/mL); Amp: Ampicillin, Gen: Gentamicin, Flu: Fluconazole

on microorganism is not fully understood, but it is speculated to involve membrane disruption by the lipophilic compounds[34]. The significant antimicrobial activity of essential oils may be due to the major chemical compounds. In this research Borneol is the major chemical compound in flower, leaf, stem of *A. odontostephana* Boiss. var. *odontostephana.* and the antimicrobial activity of this compound reported in some previous study[28,35,36]. Also for other major compound of this research reported moderate antimicrobial activity[28,37]. Despite slight activity capacities of Borneol and other compound, this compound could be responsible for the total antimicrobial activity. According to table 3, the gram-negative *Klebsiella bacteria* (PTCC 1053) had the strongest inhibitory and was the most sensitive strain than the other bacteria with MIC ranges from 32-64 µg/ml. medium inhibitory activity was against gram-negative bacteria responsible for *Escherichia coli* (PTCC 1396) and *Escherichia coli* (PTCC 1399) with MIC ranging of 32-128 µg/ml, 64-128 µg/ml, respectively. For the gram-positive bacteria *Corynebacterium glutamicum* (PTCC 1532) with the MIC values rang 32-128 µg/ml had the strongest inhibitory followed by *Staphylococcus epidermidis* (PTCC 1435) and *Staphylococcus aureus* (PTCC 1431) had the medium inhibitory activity with MIC values range from 64-128 µg/ml, 32-128 µg/ml, respectively.

Also table 3 present that all essential oils had the strong inhibitory activity with MIC values ranging 32-64 µg/ml against the all fungal strains. Hence, antifungal activity of all samples more significant than those antibacterial. All studies samples had significant antibacterial and antifungal activity. In that way, the essential oils are obtained from different parts of *Anthemis odontostephana* Boiss. var.*odontostephana.* including flower, leaf, stem and root tested for antimicrobial strains and represent an inexpensive source of natural antibacterial and antifungal substances to prevent the growth of bacteria and fungi and could be a potential medicinal resource.

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