Composition and Antibacterial Activity of the Volatile Oils from Different Parts of *Achillea tenuifolia* Lam. from Iran

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Abstract

Background: ***Achillea tenuifolia*** **Lam. (Compositae) with small yellow flowers and several times pinnately divided leaves in worm shape is known for many years in the folk medicine. It has been used to reduce sweating and to stop bleeding. It helps regulation of the menstrual cycle and reduces heavy bleeding and pain.**

Materials and Methods: Plant material (flower, leaf and stem) was collected in Khalkhal – Ardabil road area, at an altitude of 1650 m in Northwest of Iran. Plant materials were air dried 150g of flower; 150g of leaf and 200g of stem were subjected to 3h of hydrodistillation in a Clevenger-type apparatus, separately. The hydrodistilled essential oils were analyzed by GC and GC/MS methods. Antibacterial activities of the oils were evaluated by the disc diffusion method using Mueller-Hinton agar for bacteria.

Results: The oil of flower was characterized by higher amount of limonene (23.2%) and α-cadinol (18.2%). Twenty one constituents representing (92.2%) of the leaf oil were identified of which limonene (25.2%), α- pinene (14.4%), caryophyllene oxide (6.5%), α- gurjunene (6.3%), bornyl acetate (5.5%) and δ-cadinene (4.4%) were major components. The main components of the stem oil were limonene (23.6%), α- pinene (13.4%) and spathulenol (6.4%). The oils showed inhibitory effects on *Escherichia coli* and *Salmonella typhi*.

Conclusions: The main components of the oil of flower, leaf and stem were limonene, α-cadinol, α- pinene and spathulenol, but Borneol, bornyl acetate, camphor, α- and β-thujone, and 1, 8-cineol were found as the main components of essential oils of many other *Achillea* species. These variations may be attributed mainly to variation in their agroclimatic and geographical conditions. The results indicated that three oils were found to be active against bacteria, the oil from the flower was found to be more active than the oil from the leaf and stem.

Keywords: ***Achillea tenuifolia***, **Compositae, Essential oil composition, Limonene, α-cadinol**

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Introduction

The genus *Achillea* is represented in Iran by nineteen species including 7 endemics [1]. This genus is one of the most important genera of the Compositae family. *Achillea millefolium* (Yarrow) is known for many years in the folk medicine. It has been used to reduce sweating and to stop bleeding [2]. It helps regulation of the menstrual cycle and reduces heavy bleeding and pain. The main component of the essential oil of *A. millefoium* is chamazulene which has anti-inflammatory and anti-allergic properties [2].

The aqueous extract of *A. talagonica* has shown immunosuppressive activity on humoral immune system [3]. Composition of the volatile oil of the aerial parts of *Achillea wilhelmsii* C. Koch has been investigated by GLC and GC-MS. Fifty-seven components representing 98.5% composition of the essential oil were characterized. The main components of the oil were carvacrol (25.1%), linalool (11.0%), 1, 8-cineol (10.3%), E-nerolidol (9.0%) and borneol (6.4%) [4]. Chloroform extract of *A. ageratum* has shown anti-inflammatory activity on chronic and acute inflammation models and has also shown a high degree of inhibition of the Hep-2 and McCoy cells compared with 6-mercaptopurine [5, 6].

The aqueous and methanolic extracts of *A. ageratum* have exhibited analgesic and anti-inflammatory activity [7]. The essential oil of *A. fragrantissima* exerts a bactericidal effect on the Gram positive and Gram negative bacterial strains [8]. The composition of the essential oils of *Achillea clavennae* and *Achillea holosericea* has been analyzed by GC/MS. The main constituting compounds of the *A. clavennae* essential oil were camphor (41.9%) and 1,8-cineole (22.5%), while the most abundant compounds in the *A. holosericea* oil were borneol (30.2%) and camphor (14.8%). The antibacterial activity of the oil of *A. clavennae* also was tested on some microorganisms. Both examined oils showed strong activity against all tested microorganisms (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Aspergillus niger*) [9]. Hydroalcoholic extract of *A. wilhelmsii* which is wildly grows in different parts of Iran has shown antihypertensive and antihyperlipidemic activity in human [10]. The compositions of the volatile oil of *A. wilhelmsii* grown in Kerman [11], Mazandaran [12] and the constituents of the oil from Egypt and Turkey have been reported [13]. In this report the composition of the essential oils from flower, leaf and stem of *A. tenuifolia* Lam. collected from Khalkhal- Ardabil road in Northwest of Iran and those antibacterial activities are described.

Material and Methods

Plant material: Plant material (flower, leaf and stem) was collected in Khalkhal – Ardabil road area, at an altitude of 1650 m in August 2008 in Northwest of Iran. A Voucher specimen is kept at the Herbarium of Agriculture Research in Ardabil Center, Iran.

Distillation: Plant materials were air-dried in the shade prior to isolation of their oils. 150g of flower, 150g of leaf and 200g of stem of *A. tenuifolia* were collected from the wild growing plants, and subjected to 3h of hydrodistillation in a Clevenger-type apparatus. The resulting oils (yield 0.7%, 0.5% and 0.1% V/W of flower, leaf and stem respectively) were dried over anhydrous sodium sulfate and immediately placed into a dark glass tube and sealed. The samples were stored at 2°C until chemical analysis.

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GC analysis: GC analysis was performed on a Shimadzu 15A gas Chromatograph equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). N_2 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°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. Alkanes (C_8-C_{18}) were used as reference points in the calculation of relative retention indices(RRI). The relative percentages of the characterized components are given in Table 1.

GC/MS analysis: GC/MS analysis was performed using a Hewlett Packard 5973 with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm). The column temperature was kept at 60°C for 3 min and programmed to 220°C at a rate of 5°C/min and kept constant at 220°C for 5 min. The flow rate of helium as carrier gas was 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 (RI) with those given in the literature and those authentic samples [15]. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

Antimicrobial activity: Antibacterial activities of the oils were evaluated by the disc diffusion method using Mueller-Hinton agar for bacteria [16]. Discs containing 15 μL of the oils were used and growth inhibition zones were measured after 24 h of incubation at 37°C. The microorganisms used were: *Staphylococcus aureus*, *Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa.*

Results

The results obtained in the analyses of the oil of *A. tenuifolia* flower, leaf and stem are listed in Table 1, in which the percentage and retention indices of components are given. As it is shown, about 92.5% (22components), 92.2% (21 components) and 93.5% (21components) of the oils of *A. tenuifolia* flower, leaf and stem were identified, respectively. The oil of *A. tenuifolia* in different parts consisted mainly of nine monoterpenes (hydrocarbons- oxygenated) (49.5%) and thirteen sesquiterpenes (hydrocarbons- oxygenated) (43.0%) in the flower oil, also nine monoterpenes (57.2% and 54.3%) and twelve sesquiterpenes (35.0% and 39.2%) in the leaf and stem oils, respectively (Table 2). Results obtained in the antibacterial study of the essential oils are shown on Table 3. With the agar disc diffusion assay, growth inhibition was observed with 2 Gram-positive and one Gram- negative bacteria; three oils were found to be active against *Staphylococcus aureus, Enterococcus faecalis* and *Escherichia coli*. Against *S. aureus* and *E. faecalis*, the oil from the flower was found to be more active than the oil from the leaf and stem. Table 3 also shows that the oils no antibacterial activity on *P. aeruginosa*.

Discussion

The oils from flower, leaf and stem of *Achillea tenuifolia* Lam. were examined by GC and GC-MS. The constituents of the essential oils are presented in Table 1. Compositions of the oils were identified by RI and mass spectra. Thirty six compounds of the essential oils constituents were identified.

The main components of the oil of flower were limonene (23.2%), α- cadinol (18.2%), borneol (9.9%), δ- cadinene (9.0%), bornyl acetate (7.8%) and α- humulene (7.4%). Limonene (25.2%), α-pinene (14.4%), caryophyllene oxide (6.5%), α-gurjunene (6.3%), bornyl acetate (5.5%) and δ-cadinene (4.4%) were the major components in the leaf oil. The main constituents of the oil from stem were characterized by limonene (23.6%), α- pinene (13.4%), spathulenol (6.4%), α- gurjunene (6.3%), caryophyllene oxide (5.3%), bornyl acetate (5.2%), β- cubebene (4.8%) and δ- cadinene (4.3%).

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Table 1- Composition of the volatile oil of flower, leaf and stem of *Achillea tenuifolia* Lam.

Compound	RI	Percentage (%)		
		flower oil	leaf oil	stem oil
α- pinene	937	1.2	14.4	13.4
camphene	953	2.9	-	-
sabinene	975	2.3	-	-
α- phellandrene	1002	0.7	-	-
limonene	1029	23.2	25.2	23.6
3, 4- dimethylanisol	1114	-	0.2	-
trans- limonene oxide	1142	-	0.7	-
borneol	1169	9.9	2.3	2.6
γ- terpineol	1198	0.2	3.5	3.4
carone	1210	-	-	0.2
trans- carveol	1217	-	1.6	0.6
cis- carveol	1229	-	-	1.7
β- cyclocitral	1235	1.3	-	-
carvone	1243	-	3.8	3.6
bornyl acetate	1289	7.8	5.5	5.2
α- cubebene	1350	0.2	-	-
α- copaene	1377	-	0.5	0.6
β- cubebene	1388	-	3.4	4.8
α- gurjunene	1410	-	6.3	6.3
β-caryophyllene	1418	0.4	-	-
γ- elemene	1437	0.2	-	-
α- humulene	1454	7.4	-	-
γ- muurolene	1480	-	-	0.4
germacrene-d	1485	1.1	1.3	-
β-selinene	1490	0.2	-	-
β- guaiene	1493	0.5	-	-
α- selinene	1498	-	0.3	-
α – muurolene	1501	1.5	0.9	-
calarene	1514	1.1	1.2	2.1
δ- cadinene	1523	9.0	4.4	4.3
calacorene	1546	0.8	3.5	2.6
spathulenol	1578	-	-	6.4
caryophyllene oxide	1583	2.4	6.5	5.3
torreyol	1645	-	5.2	3.7
α- cadinol	1654	18.2	1.5	2.2
cadalene	1677	-	-	0.5
Total		**92.5**	**92.2**	**93.5**

Table 2- Percent of mono and sesquiterpene hydrocarbons and oxygenated compounds in flower, leaf and stem of *Achillea tenuifolia* Lam.

Compounds	Percentage (%)		
	flower oil	leaf oil	stem oil
Monoterpene hydrocarbons	30.3	39.6	37.0
Sesquiterpene hydrocarbons	22.4	21.8	21.6
Oxygenated monoterpenes	19.2	17.6	17.3
Oxygenated sesquiterpenes	20.6	13.2	17.6
Total	**92.5%**	**92.2%**	**93.5%**

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Table 3- Antibacterial activity of the essential oils from flower, leaf and stem of *Achillea tenuifolia* Lam

Microorganisms	Zone of inhibition (mm) flower oil	Zone of inhibition (mm) leaf oil	Zone of inhibition (mm) stem oil
Staphylococcus aureus	14	9	8
Enterococcus faecalis	12	10	9
Escherichia coli	8	9	7
Pseudomonas aeruginosa	NA	NA	NA

NA: Not Active

Monoterpene hydrocarbons are predominant in flower, leaf and stem oils (Table 2).

Fifty seven compounds representing 98.5 % of the essential oil from *A. wilhelmsii* aerial parts were identified. The main components of the oil were carvacrol (25.1%), linalool (11.0%), 1, 8-cineol (10.3%), E- nerolidol (9.0%) and borneol (6.4%) [4]. Nineteen components in the volatile oil of *A. wilhelmsii* from Kerman (Iran) were reported previously in which caryophyllene oxide (12.5%), camphor (9.0%), borneol (6.1%), linalool (5.5%), 1,8- cineol (3.6%), chrysanthenyl acetate (2.8%) and carvacrol (2.0%) were the main ones [11]. Camphor (2.2%) and borneol (6.4%) were present as the main components of the oil of *A. tenuifolia* reported previously [17].

The essential oils from aerial parts of *Achillea pachycephala* Rech. F. and *Achillea oxyodonta* Boiss., which are endemic to Iran, and stems, leaves and flowers of *Achillea biebersteinii* Afan., were analyzed by GC and GC-MS have reported. 1, 8-cineole (27.7%) and camphor (27.4%) as the major constituents in the oil of *A. pachycephala* and the oil of *A. oxyodonta* were characterized by higher amounts of 1, 8-cineole (38.5%) and artemisia ketone (23.0%). The oils obtained from stems and leaves of *A. biebersteinii* were rich in camphor (38.1% and 33.7%, respectively) and borneol (22.6% and 20.8%, respectively). 1, 8-cineole (13.5%) was the other main compound in stem oil. In the flower oil of the plant, camphor (36.3%) and 1, 8-cineole (22.3%) were the predominant compounds [14].

Borneol, bornyl acetate, camphor, α- and β-thujone, and 1, 8-cineol were found as the main components of essential oils of *Achillea lingulata* and *A. umbellate* [18]. These variations may be attributed mainly to variation in their agroclimatic and geographical conditions.

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