

Essential oil composition of *Achilleamillefolium* growing in Darrehshahr township

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Abstract

Achilleamillefolium belongs to the asteraceae family from genus *achillea*. In this study, essential oils were extracted from all aerial parts via hydro distillation (HD) method by clewengerset. Using the gas chromatography/mass spectrometry (GC-MS) technique causes the chemicals component of the essential oil to be identified. About 41 components, which were identified, encompassed 97.66 percent of the whole essential oil. The essential oil yields as a result of hydro distillation. After drying, about 0.56 percent of *Achilleamillefolium* was added to a balloon and then was connected to the clewenger apparatus (weight/weight) - 60 grams of plants was obtained (it has been based on dried materials). In essential oils of *Achilleamillefolium* dihydrocarveol (34.97%), the umbelulone (16.65%), 1,8-cineole (14.94%), bornyl acetate (6.08%), chrysanthenyle acetate cis (5.24%), camphene (4.21%), para-cymene (3.29%) and α -pinene (3.24%) were our major identified compounds. The purpose of this study is to identify the constituents of essential oils extracted from plants and also to determine the percentage of each compound in the essential oil of *Achilleamillefolium* used as drug.

Keywords: *Achilleamillefolium*, essential oils, clewenger, 1,8-cineole, GC-MS

Introduction

Achilleamillefolium which belongs to the asteraceae family derives from genus *achillea* with scientific name *Achilleamillefolium* L(Figure 1). The *achillea* genus has about 85 species that mostly grow in Europe, Asia and also in north America [1]. *Achillea* grows in

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west, northwest, north, northeast and center of Iran [2]. About 19 species of genus achillea have been recognized in Iran [3]. Nowadays, different medicinal functions of yarrow such as spasmolytic, choleric, treatment of wounds and anti-inflammatory activities, make it as an important medicinal plant [3]. The flour (powder) or sap of this plant is used for the treatment of various cancers and tumors of liver and mammary glands. This medicinal plant can also be used for the treatment of apoplexy, pain and cramp in heart muscle. Yarrow has components that cause decrease of bleeding, pain and inflation and we can use this components to redress the ulcers and rifts [3]. The most important medicinal usage of plant Achillea millefolium consists of treatment of wounds, inflammations, headaches, dyspepsia and spasmodic diseases [4]. Its essential oil has also anticancer, antiinflammation, antimicrobial and antioxidant properties [4,5]. Investigations of yarrow chemical composition go back to the early 1916, when Miller identified acetic acid and cineole; presently, the list of identified yarrow compounds consists of more than 120 components. It was reported that essential oil yield and its quality depends on climatic and soil conditions, genetic, plant age, phase of vegetation, anatomical part of plant and harvesting season [6]. Previously, some researches had worked on essential oils of yarrow in Iran and other countries. For example, in animals such as mice the essential oils of this plant were used for treatment of digestive ulcers and the result was very satisfactory [7]. The hypoglycemic effects of yarrow are studied on normal and diabetic mice. The results showed that the amount of glucose in diabetic mice decreased [8]. The main components found in Achillea [5,9-11], which were collected by different techniques from different parts of Iran and other countries, were presented in Table 1. Recent studies have shown that natural products and especially essential oils and components display potentials as antimicrobial agents for various medicinal uses [12,13]. The purpose of this study is to identify the constituents of essential oils extracted from plants and also to determine the percentage of each compound in the essential oil of Achillea millefolium which was used as drug. Accordingly, the type and percentage of the plant's essential oil compounds are different in each region in comparison to other regions.



Figure 1. Achilleamillefolium

Experimental

Collection of plant materials

In this study, all aerial parts of Achilleamillefolium such as stem, flowers and leaves which were used as sources to extract and identify the essential ingredients, the percentage of essential oil constituents and their surrounding areas in Darrehshahr, Ilam province were collected in June, 2011. They were kept in the shade and away from sunlight and were dried after collecting samples for days. Then, they were transferred to Lorestan University for more research.

Extraction method of essential oils

Using a Clevenger apparatus and hydrodistillation method, oil extraction plant was done. About 60 grams of the plant – after drying it – was added to a balloon and then was connected to the Clevenger apparatus. They were converted to liquid after passing through the refrigerants; then, they were collected in another container. Two-phases fluid consisting of water and oil were separated by normal hexane oil. Oil collected in the tube using a special syringe was collected after 2.5 hours. It was dehydrated with sodium sulfate. Then, it was immediately poured into the sample container in order to prevent the penetration of sunlight with aluminum foil into its close. Afterwards, to perform tests, it was kept in refrigerator.

GC analysis

GC analysis was performed by using gas chromatograph 17A Shimadzu equipped with a FID and a DB-5 capillary column (30 m×0.25 mm; 0.25 μ m film thickness). The oven temperature was programmed from 40 $^{\circ}$ C to 150 $^{\circ}$ C at 3 $^{\circ}$ C/min rate, then was held isothermal for 10 min and finally raised to 250 $^{\circ}$ C at 10 $^{\circ}$ C/min. Other operating conditions were as follow: carrier gas was Helium with a flow rate of 1.9 ml/min, injector temperature

25 °C, detector temperature 260 °C, split ratio 1:5.

GC/MS analysis

The analysis of the essential oils was performed with gas chromatography 17A shimadzu coupled with mass spectroscopy shimadzu model QP5050. Separating compounds was performed in fused silica capillary DB-5 column (30 m×0.25 mm inner diameter, with 0.25 µm film thickness). The oven temperature was programmed from 40 °C to 150 °C at 3 °C/min rate, then held isothermal for 10 min and finally raised to 250 °C at 10 °C/min. The quality of mass spectrometer was quite similar to gas spectrometer and for GC/MS detection an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium at a flow rate of 1.9ml/min. Mass range was from *m/z* 50–500 amu.

Compounds identified using the technique of GC/MS

After providing and injection of essential oil to GC system, it was the best condition to separate them. Then, by using the method of coupled gas chromatography with mass spectroscopy (GC-MS) the quantitative and quality of essential oil components were recognized. The constituent compounds of the

essential oils were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C₈-C₂₄) and the oil on a DB-5 column under the same chromatographic conditions. Identification of compounds was made by comparing their mass spectra with those of the internal reference mass spectra library data GC-MS system (wiley 229) and also with authentic compounds and it was also confirmed by our comparison of their retention indices with authentic compounds or with those of reported in the literature [14]. The relative percentage of each compound was obtained according to its under peak area in GC chromatogram, without the use of correction factors.

Results and discussion

About 41 compounds in the essential oil of aerial parts of *Achillea millefolium* were recognized using gas chromatography linked to mass spectrometry study of the composition and retention times, retention indices and mass spectra of all these parameters in comparison with standard compounds which is 97.66 percent of total oil. The oil yields were obtained by hydrodistillation 0.56 percent (w/w). In oils, the main compounds of *Achillea millefolium* were dihydrocarveol (34.97%), umbellulone (16.65%), 1,8-cineole (14.94%), bornyl acetate (6.08%),

chrysanthenyle acetate cis (5.24%), camphene (4.21%), para-cymene (3.29%) and α -pinene (3.24%). Compounds identified in Table 2 are consistent. Studies show that among different groups of terpenes, oxygenated monoterpenes has the highest concentration (Table 2). Previously, the essential oils compounds of several species of Achillea were recognized by researches from different countries. The main essential oils components were reported to include: 1,8-cineole, α -terpineol, camphor, β -pinene, borneol, bornyl acetate, γ -terpinene, terpinolene, germacren-D, cis-cahrysantenyl acetate, chamazolene and trans-nerolidol [1,5,9,16]. There are similarities in the main components of essential oils of Achillea in this study and previous studies. For example, 1,8-cineole and bornyl acetate have been reported as the main components of essential oils of species of Achilleamillefolium [1,3], both in this study and other studies. Moreover, 1,8 cineole was the main compound of the oil Achillea [1,3,5,9,11,15,16]. Comparing the present data (Table 2) with those previously reported in literature on the essential oils from Achilleamillefolium, a diversity was observed, they differ in terms of some of the main oil compounds. For example, camphor, and borneol which were found to be the major compounds in previous studies [1,5,9,16], were not detected in our work. Of course, some others derived from this main compound (such as, α -terpineol, β -pinene, γ -terpinene, terpinolene and trans (E)-nerolidol) have been presented in this study with lower percentage in essential oils (Table 2). In addition, some of the major compounds of our oil sample (dihydrocarveol, camphene, umbelulone, para-cymene and α -pinene) have not been previously reported in dominant quantity among the major compounds from the oils of Achilleamillefolium [1,5,9,16]. The antifungal [15] and antimicrobial [11] activity of the oils can be attributed to their relatively high content of oxygenated monoterpenes (such as 1,8-cineole). The most major medicinal effects of essential oils of Achillea (instance: treatment of inflammation, spasmodic diseases, dyspepsia) is due to the presence of oils' materials (dihydrocarveol, carvone, limonene, dihydrocarvone, carveol). Furthermore, these properties can be attributed to high percentage dihydrocarveole presence in oil. In conclusion, there are considerable qualitative and quantitative differences between essential oils composition of Achillea in this study, with those of previously reported from different parts of Iran and other countries. Furthermore, Chemical differentiation of Achillea essential oils might be correlated with environmental conditions, geographic, climatic, genetic, chemotypes, plant age, soil, phase of

vegetation, anatomical part of plant and from other regions, therefore, it is necessarily harvesting season [6,17-22]. Because the type important to perform many studies in this case and concentration of plant essential oils in different place of Iran for various medicinal chemical compounds in each region is different uses.

Table 1. The main components found in Achillea collected from of Iran and other countries

Entry	Region	Specie	The main compounds
1	Sivas,Turkey[5]	Achilleamillefolium subsp.	1,8-cineole, camphor, α -terpineol, β -pinene, borneol
2	Kashmir,India[9]	Achilleamillefolium L.	Camphor(28%), 1,8cineole(12%), germacrene D(12%), cis-chrysanthenyl acetate(8%)
3	Tabriz,Iran[10]	Achilleabiebersteiniifan.	Piperitone, 1,8cineole,limonene,para-cymene
		Achilleatenuifolia Lam.	γ -muurolene, α -pinene,para-cymene, camphor, trans-carveol
		Achilleafilipendulina Lam.	lim-nene, carvacrol,1, 8cineole, borneol, germacrene
4	Sivas,Turkey[11]	Achilleasetacea	1,8-cineole(18.5%)
		Achilleateretifolia	1,8-cineole(19.9%)

Table 2. Identification of essential oil compounds in the plant *Achilleamillefolium* by GC and GC/MS on DB-5 column

Entry	Name of compounds	RI	A(%)
1	Tricyclene	927	0.36
2	α -Pinene	939	3.24
3	Camphene	954	4.21
4	Verbenene	968	0.04
5	β -Pinene	979	0.09
6	Myrcene	991	0.59
7	α -Terpinene	1017	0.21
8	Para-cymene	1025	3.29
9	1,8 Cineole	1031	14.94
10	γ -Terpinene	1060	0.35
11	Cis-sabinene hydrate	1070	0.45
12	Terpinolene	1089	0.07
13	Linalool	1097	0.79
14	1-terpineole	1134	0.25
15	Cis limonene oxide	1137	0.1
16	Ment-2-en-1-ol(trans,para)	1141	0.51
17	Thujol	1169	0.81
18	Umbelulone	1171	16.65

19	α -Terpineole	1189	0.59
20	Dihydrocarveole	1194	34.97
21	Verbenone	1205	0.06
22	Bornylformate	1239	0.5
23	Chrysanthenyl acetate(cis)	1265	5.24
24	Bornyl acetate	1289	6.08
25	Lavandulyl acetate	1290	0.55
26	Pinocarvyl acetate(trans)	1298	0.06
27	Carvacrol	1299	0.17
28	Isoascaridol	1303	0.17
29	Geranyl acetate	1381	0.14
30	Isobornyl propionate	1385	0.13
31	Methyl eugenol	1404	0.02
32	Isobornylisobutanoate	1434	0.29
33	Geranylisobutanoate	1515	0.11
34	Isobornylisovalerate	1523	0.06
35	Nerolidol E	1563	0.15
36	Globulol	1585	0.02
37	Beta-copaen-4-alpha-ol	1591	0.48
38	Viridoflorol	1593	0.37
39	Beta-eudesmol	1651	0.07

40	Juniper camphor	1700	0.29
41	9-hexadecenoicacide	1880	0.19
42	Monoterpene hydrocarbons		12.45
43	Oxygenated monoterpenes		83.03
44	Sesquiterpene hydrocarbons		0.02
45	Oxygenated sesquiterpenes		1.38
46	Other compounds		0.78
	Total		97.66

RI: retention indices relative to C₈-C₂₄ n-alkanes on the DB-5column.

A: percentage of essential oils composition of achilleamillefolium

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