Original Article

Investigation of chemical composition of essential oil of stem, leaf, and flower of Achillea Tenuifolia

Marzieh Minaei[1]*, Fatemeh Minaie[2]

[1] Department of chemistry, Faculty of Basic Sciences, Qom Branch, Islamic Azad University, Qom, Iran. [2] Department of chemical engineering, faculty of Basic Technical engineering, Arak Branch, lslamic Azad University, Arak,lran.

Correspondence: Marzieh Minaei. Department of chemistry, Faculty of Basic Sciences, Qom Branch, Islamic Azad University, Qom, Iran.

ABSTRACT

The proper use of medicinal plants requires the accurate identification of the compounds in them. Due to proper climatic conditions and other factors of geographical properties, many plants are found in many regions of Iran, most of which have different properties. Essential oils of most species of yarrow are used in the health and medicinal industries. Yarrow is a plant that is most commonly found in the northwestern and northeastern of Iran, Tehran and its surroundings. In terms of medicine, this plant is very valuabl , which is collected and dried in the middle of June from the Tehran-Qom-Hassan Abad highways, whose flower, leaves, and stems were used for taking essence in Clevenger's decoction method, and then had investigated their chemical compound by analyzing GC/MS. The aim of this study was to evaluate the chemical compounds in the essential oil of *Achillea Tenuifolia* in its flower, leaf, and stem. The results of this study showed that the main compound in the flower include P-cymene 5%, Camphor 9%, 1-Terpinol 8.3%, Germacrene D 11.2%, which is humoring the pain, anti-scabies, and anti-spout. The major compound was in the leaf of 1,8-Cineol 8%, Spathulenol 12/0 %, N-octan 4%, and Camphor 5/1% and major compound in the stem include N-octane 35/1%, 3-methyl-heptane 17%, n-Decane 15% and Spathulenol 5 %.

Keywords: Chemical Compounds, Essential oil, GC/MS, Yarrow plant

Introduction

The use of herbal medicines is popular in all parts of the world, especially in developing countries; they provide the most important therapeutic needs of medicinal plants. The consumption of herbal medicines has much fewer complications than similar chemical drugs. Basically, herbal medicine is called a drug that the flower of plants is used thoroughly. The drugs and herbs we know as herbal medicines have countless compounds. These compounds, together with each other, create a very balanced state, in this situation and a critical balance, none of the compounds will be harmful, because they modulate each other's properties, and some compounds precisely are responsible to neutralize the effects of other compounds. Medicinal plants are beneficial to humans, as the general plants, but their therapeutic effects have greatly taken their place. Medicinal plants exist in different parts of the world. Today, however, due to the therapeutic effects and the profits resulting from their trade, they have been cultivated industrially and massively in different places, such as scientific and technical greenhouses, and are used in pharmaceutical factories as massive operation. Although herbal medicines do not have the effects of bad chemical drugs, they are not completely safe.

Essential oil is a viscous hydrophobic, volatile, consisting of terpene-based aromatic compounds, which are chemically

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distinct from each other and have a very strong odor of the plant that comes from it. Essences are evaporated at room temperature, so, are called volatile oils or ether oils. Essences are found in many plants. Among the most important families with essential oils, we can refer to the families of Mint, Umbellifers, Pine, Bay laurel and some plants of Rosaceae and Chicory. Essences are accumulated in some plants` tissues, such as the cellular center or in the storage site of essential oil under the trichome types, small tubers, or in the intercellular space. Typically, volatile oil are extracted from various plant organs such as flowers, buds, fruits, leaves, seeds, and so on. Extraction of this material from the plant is carried out in the following ways: (a) squeezing the plant (the epicarp part and the outer layer of the skin of Citrus fruits, especially in family Rosaceae such as raspberries, strawberries, apples and etc.); (b) use of solvents such as alcohol; (c) distillation with water and steam (boiling) like Golabgiri; (d) use of absorbent oils. In the quantity and quality of extracted essential oil, various factors are effective such as harvest time, collection method, drying and packaging, and effective maintenance. Essences are used in industries such as insecticides, preparation of aromatic gum, soap industry, toothpaste, cosmetics, perfumes, food products. With the introduction of essential oil in various ways to the body, in particular through absorption of the skin and nasal inhalation, these materials release the endorphins and enkephalin, which are protein compounds produced at the nerve endings (internal morphine), increase the threshold of pain stimulation and relieve pain and physical and mental relaxation. Essential oils` extraction methods include: distillation with water, hydrogen dispersion, and Enfleurage (an appropriate process for fresh petals with low content of essential oil such as jasmine or rose), cold pressures (a method for extracting essential oils of citrates), steam distillation (the most common and oldest method of extraction of essential oils), extraction with organic solvents, the process of microwave wave propagation (MAP) (a technique for extraction of saponins from medicinal plants) and extraction with carbon dioxide. Methods for separation and identification of essential oil compounds include fractional distillation and gas chromatography.

Achillea Tenuifolia is derived from the name of Achilles, a legendary Greek hero who used this plant in Ancient Greece to graft their soldiers' wounds. The other names of this plant are a *Ranunculus Millefoliatus*, *yarrow*, musk, shepherd, Zohreh al-Qandil and Hazenbul. This plant has a warm and dry nature. Because of the proper climatic conditions and other special geographical factors, many plants are found in most regions of Iran, most of them having different properties. *Achillea Tenuifolia* in Iran has 19 species of perennial and often aromatic herbs and is from the chicory family, its exclusive species are [1]: *aucheri. A، A –callichroa، A–oxydonta ،A– -talagonica.Akellalensis،A–eriphora، APachycephala.*

Yarrow is a plant that is most commonly found in the northwestern and northeastern Iran, Tehran and its surroundings. It is often found in deserts and ditches and infertile fields, and is a perennial plant, which is highly adapted to arid and semi-arid areas. It is a part of the Chicory family. *A. filipendulina* or *A. biebersteinii Afan* is also more popular in Azerbaijan. *A. filipendulina Lam* is mostly found in the northwest, which is valuable in terms of medicinal use. *A. Tenuifolia* has seen historically used as in traditional medicine include: pain reduction in the women's menstruation, stomach strengthener, analgesic, anti-fever, anti-hypertensive, anti-parasitic effects, especially against Ascaris cream, anti-allergy, urinary, and anesthetic, as well as its herbal tea is useful for the treatment of digestive problems [2].

Recently, secondary metabolites of medicinal herbs such as essential oils and plant extracts have been investigated for their antimicrobial effects and it has been determined that most essential oils and plant extracts extracted from medicinal plants have antifungal, antibacterial and antiviral properties. Therefore, herbal compounds have been extensively screened and used in the fields of plant pharmacology, microbiology, medical, clinical, Phytopathology, and maintaining food, fruits, and vegetables [3]. The antimicrobial activity of the essential oils is related to the phenolic compounds present in them, and many of them are categorized as harmless substances and are therefore used as antibacterial agents in food [4].

Oral consumption of essential oils causes mild irritation of mucous membranes of the mouth and the digestive tract. It also causes a feeling of warmth and increased saliva, for this reason, it is effective as an antiseptic [5]. The hydroalcoholic extract of *A. Tenuifolia* has an inhibitory effect on the secretion of the base of the stomach acid through the stomach vagus nerves. Flower branch of this plant has a tremendous effect on tympanites and reflux of food [3]. Essential oils with antioxidant compounds prevent vascular fragility and heart attacks. Epidemiologic studies showed that in patients with high levels of flavonoids in the essential oil, the risk of cardiovascular and heart disease decreases and has anti-arrhythmic effects [6].

In an investigation in 2011, essential oils of three species of Yarrow's herb in Iran and on quercetin extraction were studied. In this study, in 36 samples, the amount of quercetin in the stem of this plant was 23 ppm. Cell research has shown that quercetin helps to slow down the growth of some types of cancer cells, as well as other studies suggested that quercetin prevents cancer types such as colon cancer [7]. The general study of Nemat et al. on the genus chileaA showed that a series of compounds including terpenoids, di-terpenoids, phenylpropanoids, and carotenoids are common in this genus, and among monoterpenoids, 1,8-cineol in one-third of species and compounds with the Bornane skeleton, like camphor and borneol are the second and third commonly compounds. Essential oils are the best known active factor and a mixture of natural chemical compounds, which varies depending on the species and environmental conditions between dextrose/water (0.1-10%) [8]. Also, Saeednia et al. investigated the chemical compounds of A. Conferta Chilean essential oil in Iran, using the GC/MS method, and reported yellow essential oil with a pleasant scent and sweet taste whose odor was probably due to the camphor compounds. The results of GC/MS in this study included 48 compounds, which camphor with 22.1% and 1,8-

cineol with 10% were major compounds. In this regard, there are similarities between the two species of A. Tenuifolia and A. Conferta, which are two native species of Iran [9]. In this study, the chemical composition of essential oils of stem, leaves, and flowers of *Achillea Tenuifolia* was studied.

Experimental

Plant collection

Achillea Tenuifolia was collected from the Tehran-Qom-Hassan Abad highways in the middle of June 2013 and dried in shadows. The specimen was sent to the Forestry and Rangeland Research Institute of the Ministry of Jihad-e-Agriculture, which was identified by Dr. Mozaffarian and registered with the institute.

Extraction of essential oils from different organs of the plant

At first, all components of the plant, including flowers, leaves, and stem, were isolated and the raw and well-grounded plant material was added to a 2000 mL extraction flask together with 1200 mL of water. A Clevenger apparatus was attached to the extraction flask as well as a dropping funnel for completing the water from the distillation flask. The hydrodistillation containing the yarrow essential oil was passed through the 15 mL GC grade Ether (liquid-liquid extraction) in a collecting 25 mL pear-bottomed flask. The hydrodistillation was performed by using a thermo-controlled heating bath and the extraction process was realized up to three hours. The ether extract was then dried over anhydrous sodium sulfate, filtered and concentrated under vacuum. On the second and third days, 50 g of the stem and 40 g of leaves were weighed and the extraction steps were done like flowers. At the end of the extraction, the amount of essential oil was measured and the yield was calculated as a percent of the ratio between essential oil and raw plant masses, which was obtained as 0.2, 0.1 and 0.1 v/w%.

Separation and characterization of compounds

Separation and characterization of the essential oil contained in the plant were performed by gas chromatography. The characterization of essential oils were performed using gas chromatography attached to mass spectrometry (TRACE MS model equipped with quadrupole mass analyzer and Weston (DB-5) with a length of 30 m and an inner diameter of 0.25 mm and the thickness of the stationary phase was 0.25 μm) and compared with the standard mass spectra found in the Eight Pick Index, Nist and Firebase databases, and the GC-MS database. To confirm the characterization, the Kovats index of each compound was calculated according to its inhibition time in the gas chromatogram and the inhibition time of normal alkanes and was compared with the Kovats index of standard material. The following equation was used to calculate this index.

$$KI = 100n + 100\left(\frac{TR_x - TR_n}{TR_{(n+1)} - TR_n}\right)$$

Where KI is the coefficient of Kovats index, n is the number of carbon in the smaller hydrocarbon, TR_x is the inhibition time of the desired compound, TR_n is the inhibitory time of smaller hydrocarbon, and $TR_{(n+1)}$ is the inhibitory time of bigger hydrocarbon. It was injected 1 μL of the diluted essential oil in n-hexane to GC/MS. Temperature control of the gas chromatography (GC) is as follows: the initial temperature of the oven was 60 °C, which was fixed for 3 minutes in this temperature, then at 5 °C/min gradient, the temperature increased to 220 °C (final temperature of the oven) and was fixed for 5 minutes in this temperature.

Results and Discussion

Separation and characterization of essential oil compounds by GC/MS

The gas chromatogram from the GC-MS analysis of the yarrow flowers essential oil has been presented in Fig. 1.

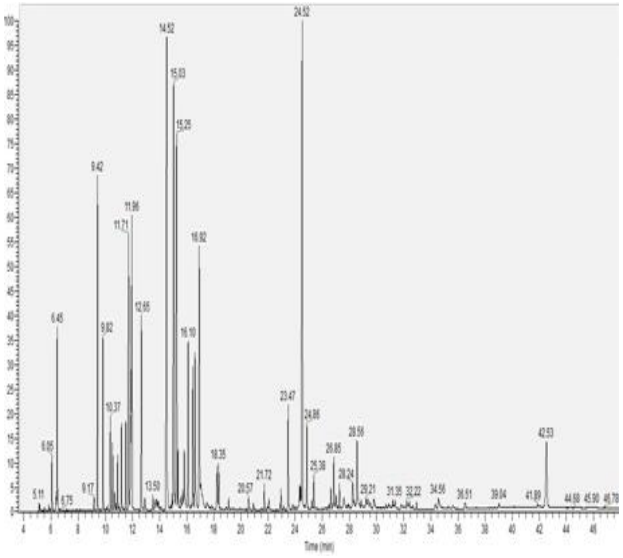

Figure 1. The gas chromatogram from the GC-MS analysis of the yarrow flowers essential oil

The gas chromatogram from the GC-MS analysis of the yarrow stem essential oil has been presented in Fig. 2.

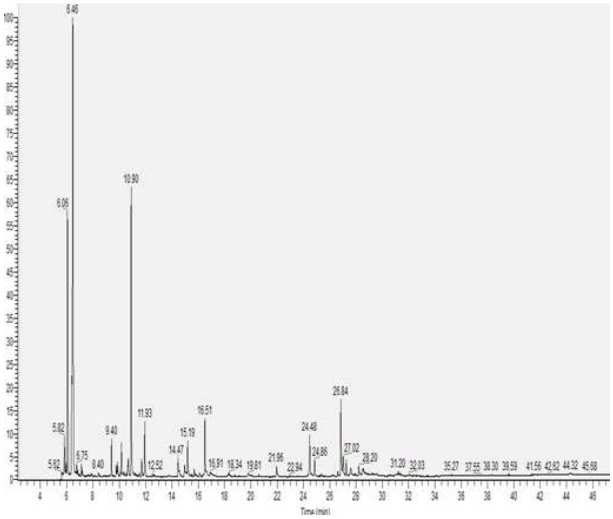

Figure 2. The gas chromatogram from the GC-MS analysis of the yarrow stem`s essential oil

The gas chromatogram from the GC-MS analysis of the yarrow leaves essential oil has been presented in Fig. 3.

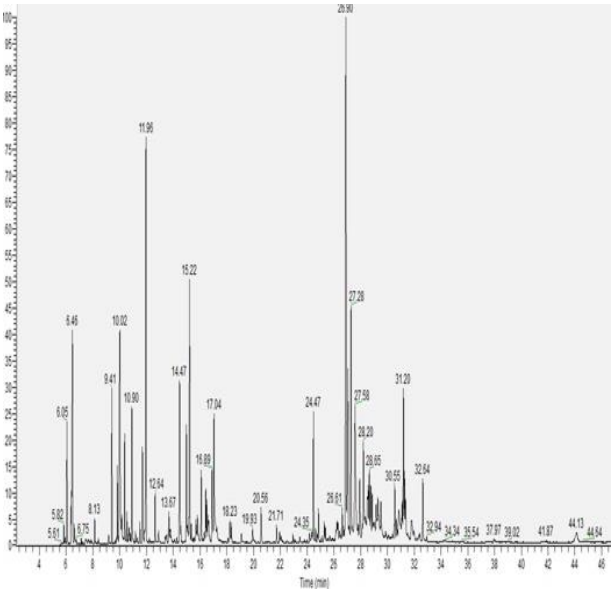

Figure 3. The gas chromatogram from the GC-MS analysis of the yarrow leaves` essential oil

The volatile components identified from the yarrow flower have been listed in Table 1 and the percentage of its group compounds has been shown.

Table 1: The main compounds identified in yarrow flowers essential oil

Entry	Flower	Area%	RI	IRREF
1	3-Methylheptane	1/0		1523
2	n-octane	3/0	800	800
3	α-Thujene	0/3	930	930
4	α-Pinene	5/2	940	939
5	Camphene	2/3	955	954
6	3-Methylnonane	0/1	969	579
7	Sabinene	1/2	977	975
8	β-Pinene	1/0	983	979
9	β-Myrcene	0/3	989	991
11	dehydro-1,8-Cineole	0/1	994	991
12	n-Decane	1/0	998	1000
13	α-Phellandrene	1/2	1008	1003
14	α-Terpinene	1/2	1020	1025
15	p-Cymene	5/0	1027	1025
16	Limonene	1/2	1033	1029
17	β-Phellandrene	2/1	1034	1030
18	1,8-Cineol	4/0	1036	1031
19	γ-Terpinene	3/0	1061	1070
20	cis-Sabinene hydrate	0/2	1070	1071
21	α-Terpinolene	0/2	1091	1091
22	Linalool	0/2	1098	1132
23	trans-Sabinenhydrate	0/2	1102	1071
24	cis-para-Menth-2-en-1-ol	12/0	1127	1122
25	1-terpineol	8/3	1145	1134
26	Camphor	9/0	1153	1146
28	cis-Chrysanthenol	0/2	1164	1164
29	Pinocarvone	0/3	1168	1165
30	Borneol	1/1	1173	1169
31	4-terpineol	3/0	1183	1177
32	α-terpineol	2/2	1195	1189
33	cis-Piperitol	3/0	1200	1196
34	trans-Piperitol	5/3	1212	1208
35	Isogeraniol	0/2	1232	
36	Piperitone	1/0	1259	1253
37	cis-Chrysanthenyl acetate	1/0	1263	1265
38	Bornyl acetate	0/1	1290	1289
39	α-Copaene	0/4	1388	1377
40	trans-Caryophyllene	0/3	1435	1419
41	Z-β-Farnesene	2/0	1456	1443
42	Germacrene D	11/2	1497	1485
43	Bicyclogermacrene	1/3	1511	1500
44	γ-cadinene	0/2	1527	1511
45	δ-Cadinene	1	1533	1530
46	1,5-epoxysalvial-4(14)-ene	0/3	1585	1548
47	Spathulenol	1/0	1594	1578
48	Caryophyllene oxide	0/2	1602	1583
49	alvia-4(14)-en-1-one	0/4	1612	1595
50	tau-Cadinol	1/0	1654	1640
51	α-Cadinol	1/2	1668	1654
52	Aromadendrene oxide-(1)	0/2	1697	1772
53	Z-α-trans-Bergamotol	0/4	1724	1691
54	n-Tricosane	2/5	2321	

The volatile components identified from the yarrow stem have been listed in Table 2 and the percentage of its group compounds has been shown.

Table 2: The main compounds identified in yarrow stem essential oil

Entry	Leaf	Area%	RI	IRREF
1	2-Methylheptane	1/0		791
2	3-Methylheptane	17/0		1523
3	n-octane	35/1	800	800
4	Tetrachloroethylene	1/0	815	826

5	n-Propylcyclopentane	1/0	834	892
6	Ethylcyclohexane	0/3	837	1678
7	o-Xylene	0/2	899	896
8	α-Pinene	2/0	939	939
9	4-Ethyloctane	0/5	953	956
10	5-Methylnonane	1/0	960	960
11	3-Ethyloctane	0/2	966	968
12	3-Methylnonane	2/0	968	579
13	β-Pinene	0/1	977	979
14	1-hexyl-3-methyl-Cyclopentane	1/0	990	
15	n-Decane	15/0	998	1000
16	α-Phellandrene	0/1	1008	1003
17	α-Terpinene	0/1	1020	1025
18	p-Cymene	1/0	1027	1025
19	Terpinene	2/4	1035	1025
20	cis-para-Menth-2-en-1-ol	1/2	1126	1122
21	α-Campholenal	1/0	1129	1126
22	1-terpineol	1/0	1143	1134
23	Camphor	2/0	1151	1146
24	Pinocarvone	0/3	1168	1165
25	4-terpineol	0/2	1182	1177
26	n-Dodecane	3/0	1197	1200
27	trans-Piperitol	0/4	1211	1208
28	cis-Chrysanthenyl acetate	0/4	1263	1265
29	n-Tetradecane	1/0	1397	1400
30	Germacrene D	3/0	1496	1485
31	Bicyclogermacrene	1/0	1511	1500
32	1,5-epoxysalvial-4(14)-ene	0/2	1585	1548
33	spathulenol	5/0	1594	1575
34	Caryophyllene oxide	1/3	1601	1583
35	salvial-4(14)-en-1-one	1/0	1612	1595
36	Ledene oxide-(II)	1/0	1627	
37	Isopatchoulane	1/0	1653	1631
38	tau-Cadinol	0/4	1668	1640

The volatile components identified from the yarrow leaves have been listed in Table 3 and the percentage of its group compounds has been shown.

Table 3: The main compounds identified in yarrow leaves essential oil

Entry	Stem	Area%	RI	IRREF
1	3-Methylheptane	2/0		1523
2	n-octane	4/0	801	800
3	cis-3-Hexenal	0/3	808	784
4	α-Thujene	0/1	930	930
5	α-Pinene	2.1	939	939
6	Camphene	1/0	955	954
7	Benzaldehyde	7/3	963	960
8	3-Methylnonane	0/3	969	579
9	Sabinene	2/0	977	975
10	β-Pinene	0/4	983	979
11	dehydro-1,8-Cineole	0/1	994	991
12	n-Decane	2/2	998	1000
13	α-Phellandrene	1/0	1008	1003
14	α-Terpinene	0/3	1020	1025
15	p-Cymene	2/0	1027	1025
16	Limonene	0/3	1032	1029
17	1,8-Cineol	8/0	1036	1031
18	γ-Terpinene	1/0	1061	1070
19	cis-Sabinene hydrate	0/2	1070	1071
20	α-Terpinolene	0/2	1091	1091
21	Linalool	0/1	1097	1132
22	trans-Sabinene hydrate	0/4	1101	1098
23	cis-para-Menth-2-en-1-ol	3/0	1126	1122
24	1-terpineol	2/0	1144	1134
25	Camphor	5/1	1152	1146
26	cis-Chrysanthenol	0/1	1164	1164
27	Pinocarvone	0/4	1168	1165
28	Borneol	0/5	1172	1169
29	4-terpineol	1/2	1182	1177
30	4Z-Decenal	0/2	1191	1390
31	α-terpineol	1/0	1194	1189
32	n-Dodecane	1/0	1197	1200
33	cis-Piperitol	0/5	1200	1196
34	trans-Piperitol	2/0	1211	1208
35	Fragrano	3/3	1216	1216
36	Piperitone	0/5	1259	1253
37	cis-Chrysanthenyl acetate	0/3	1263	1265
38	Bornyl acetate	0/1	1290	1289
40	α-Copaene	0/3	1387	1377
41	n-Tetradecane	0/14	1396	1400
42	trans-Caryophyllene	0/2	1435	1419
43	E-β-Ionone	0.3	1491	1489
44	Germacrene D	2/1	1495	1485
45	epi-Cubebol	0/2	1507	1495
46	Bicyclogermacrene	1/0	1511	1500
47	10-epi-Cubebol	0/4	1529	1535
48	δ-Cadinene	0/2	1533	1530
49	1,5-epoxysalvial-4(14)-ene	1/0	1584	1548
50	Spathulenol	12/0	1596	1578
51	Caryophyllene oxide	4/0	1602	1583
52	salvial-4(14)-en-1-one	4/0	1613	1595
54	Ledene oxide-(II)	1/3	1640	1646
55	Isopatchoulane	2/0	1653	1631
56	β-Eudesmol	1/0	1668	1616
58	4-isopropenyl-3-Carene	1/0	1677	1629
59	4-Oxo-β-ionone	1/0	1681	1661
60	Mustakone	1/0	1694	1653
64	14-oxy-alpha-Muurolene	1/1	1773	1770
66	τ-cadinene aldehyde	1/1	1792	1505
68	14-hydroxy-delta-Cadinene	1/2	1818	1807

70	n-Tetracosane	1/0	2379	366

Comparison of the chemical compounds of the *Achillea Tenuifolia* essential oil has been presented in Table 4.

Table 4: Comparison of the chemical compounds of *Achillea Tenuifolia* essential oil

Number	Chemical compounds	IREXT	$_{REF}$IR	Flower AREA %	Leaf AREA %	Stem AREA %
1	3-Methylheptane		1523	1	2	1
2	n-octane	800	800	3	4	35/1
3	α-Pinene	940	939	5/2	2/1	2
4	3-Methylnonane	969	579	1	0/3	2
5	β-Pinene	983	979	1	0/4	0/1
6	n-Decane	998	1000	1	2/2	15
7	α-Phellandrene	1008	1003	1/2	0/2	1
8	p-Cymene	1027	1025	5	2	1
9	cis-para-Menth-2-en-1-ol	1127	1122	12	3	½
10	1-terpineol	1145	1134	8/3	2	1
11	Camphor	1153	1146	9	5/1	2
12	Pinocarvone	1168	1165	0/3	0/4	0/3
13	4-terpineol	1183	1171	3	2	1
14	trans-Piperitol	1212	1208	5/3	2	0/4
15	cis-Chrysanthenyl acetate	1263	1265	1	0/3	0/4
16	Germacrene D	1497	1485	11/2	2/1	3
17	Bicyclogermacrene	1511	1500	1/3	1	1
18	1,5-epoxysalvial-4(14)-ene	1585	1548	0/3	1	0/2
19	Spathulenol	1594	1578	1	12	5
20	Caryophyllene oxide	1602	1583	0/2	4	1/3
21	salvial-4(14)-en-1-one	1612	1595	0/4	4	1

By comparing the main compounds of *Achillea Tenuifolia* essential oil with some species of *Achillea Tenuifolia*, it was found that there was a lot of similarities between them. The main compounds of flower, leaf, stem essential oil, including Camphor, 1,8-Cineol, Germacrene D, α-pinene, which can be expected to have similarities in properties.

Conclusion

Due to the main compounds of *Achillea Tenuifolia,* the essential oil from its flower, leaf, and stem, it can be said that it has many medicinal properties, but more research is needed. Camphor is one of the main components that is used in the health and beauty industries and as an important economic source in the chemical and pharmaceutical industries. Also, 1,8-Cineol used in anticough. Linalool in the flower and leaf has antimicrobial properties. γ-Terpinene in the flower and leaf also has the oxidative properties.

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