World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Determination of essential oil components of *Achillea millefolium*

Cenk Paşa [1,*] and Turgut Kılıç [2]

[1] *Balikesir University, Altinoluk Vocational School, Department of Plant and Animal Production, Medicinal and Aromatical Plants Programme, Edremit, Balikesir, Türkiye.*
[2] *Balikesir University, Necatibey Education Faculty, Department of Science Educations, Balikesir, Türkiye.*

World Journal of Advanced Research and Reviews, 2024, 24(03), 1496–1502

Publication history: Received on 17 October 2024; revised on 14 December 2024; accepted on 16 December 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.24.3.3611

Abstract

Medicinal plants used in traditional medicine are being investigated and employed more and more in the food, pharmaceutical, and nutraceutical industries these days. Achillea sp. which is in the Asteraceae family is one of the most widely used medicinal and aromatic plants for the treatment of various diseases. Both traditional medicine and modern medicine make extensive use of yarrow (*Achillea millefolium* L.). The objective of this study was to ascertain the essential oil content and essential oil constituents of *Achillea millefolium* species, which are found in Ulus Mountain in the Sındırgı district of Balıkesir province. The components of the essential oil were examined using a GC-MS device after the hydrodistillation process as used to obtain it. The analysis revealed a significant concentration of the carvacrol component (10.16 %) in the essential oil of the *Achillea millefolium* plants. A total of 95 components were identified in *A. millefolium* essential oil which accounted for 89.95 %. Carvacrol (10.16 %), γ-Muurolene (9.58 %), Grandisol (5.28 %), Artemisia ketone (3.70 %) were found as major constituents of *A. millefolium* essential oil.

Keywords: Achillea millefolium; Carvacrol; γ-Muurolene; Essential oil; GC-MS

1. Introduction

There are over 140 endemic species in the genus Achillea (Asteraceae), which is found throughout Europe and the Middle East. The Turkish flora contains 40 Achillea sp., 20 of which are endemic. This genus is widely distributed around the world. Additionally, it has been used traditionally for a variety of purposes, including wound healing, hemorrhoids, diarrhea, and abdominal pain. Moreover, Achillea sp. is commonly used as food (Öztürk et al.,2023).

Achillea millefolium L, commonly referred to as "Yarrow," has been used in Turkey as a diuretic, appetite enhancer, menstrual bleeding remedy, and wound healer. There are several ways to use the plant's aerial components, such as infusion, tincture, liquid extract, entire extract, and bath. The plant's essential oil has also been used to treat mouth sores and promote dental health because of its antibacterial properties. It's also the preferred treatment for oral wounds and aphtha (Öztürk et al.,2023).

Achillea millefolium is a perennial herbaceous species from the Asteraceae family and is considered one of the oldest medicinal plants. This species, which varies between 30-90 cm in height, is covered with dense hairs. The leaves of yarrow, which has a unique scent, are hairy. Its white flowers are in the form of a large anther and consist of tubular and ligulate flowers. Its seeds are small and hard and round in structure. These seeds do not have hairs called pappus. It is used for indigestion and colds among the public. The dried flowering parts of this plant are used medicinally. This plant, which has a slight odor and a bitter taste, contains volatile oil, sesquiterpenes, flavonoids and tannins. The composition and amount of the bluish colored volatile oil vary greatly depending on the variety of the plant and the time

* Corresponding author: Cenk Paşa

and region of extraction. The herb part usually contains 0.2-0.4 % volatile oil. This essential oil contains azulene, limonene, cineole, borneol, pinenes and sesquiterpenes (Baytop, 1999).

The flowers (Flos Millefolii) and the herb (Herba Millefolii) are the plant parts that are utilized. According to Marquard and Kroth (2001), yarrow is used to cure wounds, relieve cramps, diuretics, aperitifs, carminatives, and irregular menstruation (Karamenderes and Kesercioğlu, 2002). The type of plant, when it is obtained, and the location all have a significant impact on the essential oil's composition and concentration (Baytop, 1999). The German Codex states that the minimum essential oil rate in Herba Millefolii is 0.2 % (0.5 % in the flower, 0.2-0.7 % in the stem and leaves), and that there are various chemotypes based on the plant and that the active ingredient composition varies greatly (Marquard and Kroth, 2001).

According to previous studies, the primary constituents of *Achillea millefolium* species' essential oil include mono- and sesquiterpenes. α-Pinene, β-pinene, β-phellandrene, α-thujane, α-terpinene, and γ-terpinene were found in the plant's essential oil (Sevindik et al., 2016; Mazandarani et al., 2013). They also found the monoterpenes camphene, limonene, and sabinene (Kazemi 2015; Nadim 2011). The *Achillea millefolium* species was shown to include (E)-β-caryophyllene, β-cubebene, and germacrene-D sesquiterpene components by Costescu et al. (2014) and Sevindik et. al. (2016).

The growth season, geographic location, soil type, harvest time, various drying techniques, and distillation procedure all affect the chemical content of plants (Baydar et al., 2004).

The purpose of this study was to identify the essential oil constituents of *Achillea millefolium* species that were collected from Ulus mountain in the Balıkesir province's Sındırgı district.

2. Material and methods

In 2023, samples of *Achillea millefolium* were taken from Ulus Mountain in the Sındırgı region of Balıkesir, which is 1600 meters above sea level. Prof. Dr. Selami Selvi identified the species of the samples that were collected. The plant material that was gathered was allowed to dry at room temperature (18°C). Using a Clevenger-style device, 100 g of plant material was hydrodistilled for three hours to extract essential oil from dried samples. Until the subsequent analysis, the extracted essential oils were kept in a refrigerator at 4°C.

Sample Preparation; 20 uL of extract was dissolved in 1480 uL dichloromethane, prepared solution was given to analysis. The GC-MS analysis was carried out with Thermo Scientific Trace GC 1300 connected to Thermo TSQ 9610 MS-MS system on a DB-5 (Thermo Scientific TG-5MS) capillary column (30 m x 0.25 mm, 0.25 um film thickness) was used with helium as carrier gas (1.0 mL/min). GC oven temperature was kept at 50°C for 1 min and programmed to 280°C at a rate of 10°C/min, and kept constant at 280°C for 2 min. Total analysis duration is 26 minutes with solvent cut-off set for 4 minutes. Split ratio was adjusted at 30:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 650.

3. Results and discussion

By using GC-MS, the constituents of *Achillea millefolium* essential oils were evaluated and investigated. The analysis revealed a significant concentration of the carvacrol component (10.16 %) in the essential oil of the *Achillea millefolium* plants. A total of 95 components were identified in *A. millefolium* essential oil which accounted for 89.95 %. Carvacrol (10.16 %), γ-Muurolene (9.58 %), Grandisol (5.28 %), Artemisia ketone (3.70 %) were found as major constituents of *A. millefolium* essential oil. The other constituents were given in Table 1.

Table 1 The essential oil of *Achillea millefolium* contains volatile components.

R.T.*	Compounds	%
7.61	ß-Pinene	0.12
7.98	Benzene, 1-ethyl-3-methyl-	0.30
8.05	3,3,6-Trimethyl-1,4-heptadien-6-ol	0.85
8.30	3-Cyclohexene-1-carboxaldehyde, 2,4,6-trimethyl-	0.11
8.72	p-Cymene	2.08

8.83	Limonen	0.14
8.90	Eucalyptol	2.17
9.57	Artemisia ketone	3.70
10.11	2,6-Octadien-4-ol, 2,7-dimethyl-	0.23
10.26	Cyclohexene, 3-(3-methyl-1-butenyl)-, (E)-	1.16
10.50	Linalool	2.91
10.61	Hotrienol	0.35
10.65	Chrysanthenone	0.61
10.82	Tetrahydrofuran, 2-isobutenyl-4-vinyl-	0.11
10.97	3-Cyclohexene-1-carboxaldehyde, 2,4,6-trimethyl-	0.15
11.04	Isophorone	0.62
11.18	1,7,7-Trimethylbicyclo[2.2.1]hept-5-en-2-ol	0.17
11.53	Pinocarveol	1.29
11.65	Verbenol	0.39
11.75	Cyclohexene, 3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl-	0.19
12.06	cis-Verbenol	1.99
12.13	Lavandulol	0.74
12.18	Borneol	1.11
12.23	2-Octen-4-ol, 2-methyl-	0.13
12.44	Terpinen-4-ol	0.43
12.54	2-Isopropylidene-3-methylhexa-3,5-dienal	0.11
12.59	p-Cymen-8-ol	0.30
12.65	5-Caranol, trans,trans-(+)-	0.13
12.75	α-Terpineol	0.42
12.90	Myrtenol / Homomyrtenol	0.54
13.21	Verbenone	0.50
13.30	Grandisol	5.28
13.40	Carveol	0.43
13.91	Cuminal	0.19
14.03	Carvone	0.21
14.24	3-Cyclohexen-1-one, 2-isopropyl-5-methyl-	1.81
14.38	Bicyclo [3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate	3.66
14.84	2-(1,4,4-Trimethylcyclohex-2-en-1-yl)ethyl p-toluenesulfonate	0.21
14.99	Lavandulol acetate	2.11
15.26	Carvacrol	10.16
15.50	Verbenone acetate	0.16
15.64	Verbenone	0.14
15.75	1,3-Cyclopentadiene, 5,5-dimethyl-1-ethyl-	0.43

15.82	1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-	0.11
15.85	1,6-Dimethylhepta-1,3,5-triene	0.10
16.23	Spiro[4.5]decane, 6-methylene-	1.81
16.96	Neryl propionate	0.22
17.51	Benzenebutanal, γ,4-dimethyl-	0.12
17.87	Farnesol	0.14
18.27	Cyclobutane, 1,2-bis(1-methylethenyl)-, trans-	0.25
18.81	β-Chamigrene	0.60
19.13	β-Chamigrene	0.46
19.20	α-Curcumene	0.94
19.26	Farnesene epoxide, E-	0.19
19.32	Limonen-6-ol, pivalate	0.11
19.51	Adamantane, 1,3-dimethyl-	0.87
19.56	Ledene	0.05
19.69	Neryl (S)-2-methylbutanoate	0.79
20.08	Lanceol, cis	0.43
20.40	Isolongifolene, 4,5,9,10-dehydro-	0.27
20.49	Cadala-1(10),3,8-triene	0.17
20.71	γ-Gurjunenepoxide-(1)	0.63
20.81	Humulene-1,2-epoxide	0.21
20.89	Calarene epoxide	0.10
20.99	1-Isopropenyl-3-propenylcyclopentane	0.33
21.05	γ-Elemene	0.76
21.12	p-Menthane, 2,3-dibromo-8-phenyl-	0.73
21.24	α-Guaiene	2.77
21.35	Aromadendrene oxide-(1)	0.24
21.44	α-Santalol	0.79
21.51	Epiglobulol / Veridiflorol	2.75
21.67	Cedren-13-ol, 8-	0.14
21.74	Epiglobulol / Veridiflorol	0.78
21.80	α-Cadinol	1.68
21.93	Caryophyllene oxide	0.14
22.03	α-acorenol	0.24
22.07	Cedren-13-ol, 8-	0.55
22.15	Ledene oxide-(II)	2.59
22.28	γ-Muurolene	9.58
22.34	β-Cedrene	0.11
22.46	β-Cedrene	0.18

22.61	trans-Nuciferol	3.21
22.78	α-acorenol	0.38
22.86	ß-bisabolol	0.10
22.93	1-Isopropenyl-3-propenylcyclopentane	1.16
23.03	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	0.73
23.12	geranyl-α-terpinene	0.43
23.18	Aromadendrene oxide-(2)	0.10
23.24	Ledene oxide-(II)	0.28
23.32	Aromadendrene oxide-(2)	0.14
23.55	ß-Vatirenene	0.19
23.82	9H-Cycloisolongifolene, 8-oxo-	0.62
24.35	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	0.15
24.87	Palustrol	0.51
25.15	9H-Cycloisolongifolene, 8-oxo-	0.19
	Total	89.95

*Retention time

The chemical components of *Achillea millefolium* essential oil have been the subject of many studies. These investigations have shown that the maturation of the plant with increasing levels of monoterpenes due to sesquiterpenes is associated with changes in the composition of *Achillea millefolium* essential oil. In many additional Achillea species, the primary essential oil components have been identified as 1,8-cineole, camphor, and α-terpineol (Rohloff et al., 2000).

According to research done in Russia and Cuba, the *Achillea millefolium* plant's essential oil had high levels of β-caryophyllene and camazulen, as well as high levels of sesquiterpenes (Orav et al., 2006; Ahmadi et al., 2017). 43 volatile chemicals were found in a study that used GC-MS to identify the chemical composition of essential oils. The study also assessed the essential oils antibacterial and antioxidant properties. Camphor (12.8 %), germacrene (12 %), nerolidol (7.3 %), sabinene (6.7 %), and 1,8-cineole (4 %) were the principal constituents (El-Kalamouni et al., 2017).

In a similar study, *Achillea millefolium* essential oil content and composition were evaluated. According to reports, the plant's essential oil content was 0.70 % ± 0.05. According to Nadim et al. (2011), the principal constituents were sabinene (17.58 %), 1,8-cineole (13.04 %), borneol (12.41 %), bornyl acetate (7.98 %), β-pinene (6.28 %), terpinene, and camazulen (5.28 %). The primary constituents identified in the Kazemi et al. (2015) study were carvacrol (10.14 %), borneol (16.35 %), limonene (14.53 %), and thymol (26.47 %). According to Almadiy et al. (2016), the primary constituents of *A. millefolium* essential oil were chamazulene (26.2 %), β-pinene (16.6 %), sabinene (9.2 %), germacrene D (6.7 %), and β-caryophyllene (5.9 %). When Candan et al. (2003) examined the essential oil of *A. millefolium*, they found that the main constituents were 1,8-cineole (24.6 %), camphor (16.7 %), α-terpineol (10.2 %), β-pinene (4.2 %), and borneol (4.0 %). 102 compounds were found by Orav et al. (2005) in 19 distinct *Achillea millefolium* essential oils that were cultivated in various parts of Europe. The primary constituents of the different essential oils of *A. millefolium* were identified as chamazulene (0-42.0 %), α-thujone (0-26.6 %), camphor (0.1-24.5 %), β-bisabolol (0-21.6 %) and β-pinene (0-20.3 %). The primary constituents identified by Zöngür (2023) in his study conducted in the Sivas region were 1,8-cineole (19.33 %), α-phelandrene (12.47 %), camphor (11.87 %), and kamazulene (12.22 %).

The sesquiterpene fraction in the essential oil of *Achillea millefolium* of Turkish origin was found in appreciable amounts, but was found to be qualitatively different from that reported above.

4. Conclusion

Achillea millefolium is a useful plant that is used in medicine to treat skin conditions, mouth infections, colds and dyspeptic illnesses. Its biological activity and traditional usage have earned it a significant position. As a result, it is now crucial to describe the plant's chemistry and determine its suitability for human consumption. Among the 95 components we found in the *Achillea millefolium* plant, the two most important ones were carvacrol (10.16 %) and γ-muurolene (9.58 %). Our research yielded datas that is comparable to existing literature and is believed to be useful for studies on medicinal plants, pharmaceuticals, and biodiversity.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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