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Research Article

Evaluation of volatile components of *Achillea millefolium* L. essential oil

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ABSTRACT

The genus *Achillea* (Asteraceae) is distributed in Europe and the Middle East and has approximately 140 endemic species. There are 40 *Achillea* sp. in the Turkish flora and 20 of them are endemic. This genus has a widespread area all over the world. Also, it has many different uses in traditionally, such as diarrhea, abdominal pain, hemorrhoids and wound healing. *Achillea* sp. is also widely used as food.

Achillea millefolium L. is known as "Yarrow" and has been used as a wound healer, diuretic, appetite stimulant and menstrual bleeding in Türkiye. The aerial parts of the plant are used in a variety of forms, including infusion, tincture, liquid extract, total extract, bath. It has also been stated that the essential oil of the plant is used in mouth sores and dental health due to its antimicrobial effect. It is also preferred in the treatment of aphtha and wounds in the mouth.

Within the scope of this project, essential oil of *A. millefolium* was obtained from European Pharmacopoeia (9th Edition) quality, supplied from German commercial source, with the Clevenger apparatus for 3 h. The chemical composition of the essential oil obtained was elucidated.

A total of 67 components were identified. Chamazulene (6.8%), caryophyllene oxide (5.8%), torilenol (5.6%), (*E*)-nerolidol (4.3%), borneol (4.0%) were found as major constituents of *A. millefolium* essential oil.

The original value of this study, aim of this study is to conduct a pharmacognosic examination of the European Pharmacopoeia quality *A. millefolium*, one of the *Achillea* sp. that is of great importance worldwide due to medicinal properties, especially herbal tea. With this study, the chemical composition of the volatile components of the *A. millefolium* was elucidated.

Keywords: Asteraceae, *Achillea millefolium* L., essential oil, GC, GC-MS

1. INTRODUCTION

Achillea millefolium L. is a perennial herbaceous species from the Asteraceae and it is considered one of the oldest medicinal plants. It is an erect herbaceous perennial plant that grows up to 50 cm tall, with a slender cropping rootstock throwing numerous roots and stolons with a blunt, succulent scale at each node. The leaves are 5–20 cm long, bipinnate or tripinnate, almost feathery, having varying degrees of hairiness (pubescence) and arranged spirally near the middle and bottom of the stem. The flowers are

typically white, but either pink or pale purple flowers with corymbose, ovoid, flat-topped heads at the end of stems and branches, having densely arranged petals in flattened clusters. Fruits are 2-mm, shiny, oblong achenes, with broadly winged margins and no pappus [1, 2]. *A. millefolium* can grow even at an altitude of 3500 meters above sea level. 130 species have been identified in Europe, Asia and America, 40 species in Türkiye and 19 species in Iran. It is one of the oldest known herbal medicines. It grows in clusters in sunny and warm environments, on narrow field paths, roadsides, meadows and sunny slopes. Flowering is observed between May and October, the flowers are generally white [3, 4]. It is popularly used for indigestion and colds. Dried flowering parts of this plant are used medicinally [1].

The phytochemical proporties of the *A. millefolium* has been revealed through studies conducted for many years. The various flavonoids such as apigenin, quercetin, kaempferol, naringenin, rutin and acacetin etc. [5-8]; phenolics such as *p*-coumaric acid, caffeic acid, ferulic acid etc. reported from different parts of *A. millefolium* [7-10]. In addition, mono- and sesquiterpenes reported as a major components from *A. millefolium* essential oils. *α*-Pinene, *β*-pinene, *β*-phellandrene [11], *α*-thujane, *α*-terpinene and *γ*-terpinene [12], camphene and limonene [13-14], and sabinene [14-16] are the monoterpenes identified in *A. millefolium* essential oil. (*E*)-*β*-caryophyllene [11, 15], *β*-cubebene, germacrene-D [17], are the sesquiterpenes hydrocarbons found in *A. millefolium* essential oils. Also, chamazulene which gives the its characteristic color-bluish [17] contains in *A. millefolium* essential oils.

A. millefolium is used as wound healer, diuretic, carminative, menstrual regulator, prevent stomach problems such as gastritis and ulcers, kidney stone reducer, blood and liver cleanser, anti-inflammatory, headache reliever and prostate treatment traditionally [1, 18]. Its has wide range of pharmacological activities such as antimicrobial [2], antihelmintic [19], antioxidant [20], antiinflammatory [21], antispazmodic [22], anticancer [23], antiulcer [24], hepatoprotective [25] activities.

In this study, it was aimed to reveal the phytochemistry of *A. millefolium* essential oil and its quality.

2. MATERIALS AND METHODS

2.1. Material

Pharma grade dried *A. millefolium* was obtained from German commercial source (Caesar & Loretz GmbH). Essential oil was obtained by hydrodistillation for 3 h with Clevenger apparatus. The yield of essential oil was found %0.025.

2.2. GC and GC-MS analyses

The essential oil was analyzed by GC using a Hewlett Packard 6890 system (SEM Ltd, Istanbul, Turkey) and an HP Innowax fused silica capillary column (FSC) (60 m x 0.25 mm Ø, with 0.25 µm film thickness) was used with nitrogen at 1 mL/min. Initial oven temperature was 60°C for 10 min, and increased at 4 °C/min to 220 °C, then kept constant at 220 °C for 10 min and increased at 1 °C/min to 240 °C. Injector temperature was set at 250 °C. Percentage compositions of the individual components were obtained from electronic integration using flame ionization detection (FID, 250 °C) (Demirci vd., 2015). Relative percentages of the separated compounds were calculated from FID chromatograms as cited in Table 1 [26].

GC-MS analysis was performed with a Hewlett-Packard GCD, system (SEM Ltd, Istanbul, Turkey) and Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with Helium. GC oven temperature conditions were as described above, split flow was adjusted at 50 mL/min, the injector temperature was at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 425 as previously reported [26].

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC-MS Library, MassFinder

4.0 Library) [27, 28], and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data [29] was used for the identification as also previously reported in detail [26].

3. RESULTS AND DISCUSSION

A. millefolium essential oil was analyzed by GC and GC/MS systems, simultaneously. A total of 67 components were identified in A. *millefolium* essential oil which accounted for 70.7%. Chamazulene (6.8%), caryophyllene oxide (5.8%), torilenol (5.6%), (*E*)-nerolidol (4.3%), borneol (4.0%) were found as major constituents of *A. millefolium* essential oil. The other constituents were given in Table 1.

When the compared the EP (9th edition), the standard for content of proazulenesis not less than 0.02%, expressed as chamazulene, calculated to the dried drug [30]. Our results were confirmed the quality of essential oil.

Many researchers investigated the chemical compositions of *A. millefolium* essential oil. In previous work of Kazemi et al. (2015), the major constituent was thymol (26.47%), followed by borneol (16.35%), limonene (14.53%), carvacrol (10.14%) and *α*-pinene (10.12%) [20]. Almadiy et al. (2016) were chamazulene (26.2%), *β*-pinene (16.6%), sabinene (9.2%), germacrene D (6.7%), and *β*-caryophyllene (5.9%) were the main compounds in *A. millefolium* essential oil [31]. Candan et al. (2003), analyzed the *A. millefolium* essential oil and 1,8-cineole (24.6%), camphor (16.7%), *α*-terpineol (10.2%), *β*-pinene (4.2%), and borneol (4.0%) were found as major components [32]. According to Jianu et al. (2016), chamazulene (16.37%) and germacrene D (15.38%) were found as the main components in the *A. millefolium* essential oil, the yield being 0.43% (v/w) [33]. Orav et al. (2005) identified 102 compounds from 19 different *A. millefolium* essential oils, grown in different regions of Europe. 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 %) were found as main components in different *A. millefolium* essential oils [34].

Table 1. Volatile components of *Achillea millefolium* L. essential oil

RRI[a]	Compound	%[b]
1032	*α*-Pinene	0.1
1035	*α*-Thujene	0.1
1093	Hexanal	0.1
1118	*β*-Pinene	0.2
1163	Isomenthone	0.1
1188	*α*-Terpinolene	0.1
1203	Limonene	trc
1213	1,8-Cineole	0.9
1244	2-Pentyl furane	0.2
1255	*γ*-Terpinene	0.2
1280	*p*-Cymene	0.5
1358	Artemisia ketone	0.2
1403	Yomogi alcohol	0.4
1430	*α*-Thujone	0.1
1450	2,5-Dimethyl styrene	tr
1451	*β*-Thujone	0.1
1452	*α,p*-Dimethylstyrene	0.1
1497	*α*-Copaene	0.2
1532	Camphor	2.8
1538	*trans*-Chrysanthenyl acetate	0.5
1553	Linalool	0.4
1586	Pinocarvone	0.3
1590	Bornyl acetate	0.6
1611	Terpinen-4-ol	1.3
1612	*β*-Caryophyllene	1.0
1648	Myrtenal	0.6
1706	*α*-Terpineol	1.3
1658	Sabinyl acetate	0.5
1664	*trans*-Pinocarveol	0.4
1709	*α*-Terpinyl acetate	1.0
1719	**Borneol**	**4.0**
1748	Piperitone	0.1
1764	*cis*-Chrysanthenol	0.3
1773	*δ*-Cadinene	0.5
1776	*γ*- Cadinene	0.3
1786	*ar*-Curcumene	0.1
1845	*(E)*-Anethol	1.4
1804	Myrtenol	0.2
1827	*(E,E)*-2,4-Decadienal	0.9
1868	*(E)*-Geranyl acetone	0.4
1941	*α*-Calacorene	0.4

RRI[a]: Relative retention indices calculated against *n*-alkanes;
%[b]: calculated from the FID chromatograms;
tr[c]: trace amount (< 0.1%).

Table 1. Continued

RRI[a]	Compound	%[b]
1945	1,5-Epoxy-salvial-4(14)-ene	0.3
1948	*trans*-Jasmone	0.4
1958	*(E)-β*-Ianone	0.2
2008	**Caryophyllene oxide**	**5.8**
2037	Salvial-4(14)-en-1-one	0.6
2050	***(E)*-Nerolidol**	**4.3**
2071	Humulen epoxide-II	0.6
2092	*β*-Oplopenone	1.6
2104	Viridifrolol	2.4
2131	Hexahydro farnesyl acetone	1.1
2144	Spathulenol	2.8
2179	3,4-Dimethyl-5-penthylidene-2(5H)-furanone	0.6
2187	*T*-Cadinol	1.3
2232	*α*-Bisabolol	0.7
2250	*α*-Eudesmol	0.1
2255	*α*-Cadinol	3.0
2257	*β*-Eudesmol	0.5
2265	Longiverbenone	0.5
2273	Selinen-11-en-4 *α*-ol	2.2
2278	**Torilenol**	**5.6**
2298	Decanoic acid	2.0
2300	Tricosane	0.7
2316	Caryophylladienol I	1.2
2400	Tetracosane	0.8
2430	**Chamazulene**	**6.8**
2600	Hexacosane	1.7
	Total	**70.7**

RRI[a]: Relative retention indices calculated against *n*-alkanes; %[b]: calculated from the FID chromatograms; tr[c]: trace amount (< 0.1%).

4. CONCLUSION

A. millefolium is used in the treatment of colds, dyspeptic diseases, mouth infections, and skin diseases. It also has many valuable biological activities such as antimicrobial, antioxidant and anticancer. Thanks to its traditional uses and biological activities, *A. millefolium* has an important place. Therefore, it has become important to elucidate its chemistry and reveal its quality for consumption. In conclusion, our results as found as similar in literature. It was confirmed that essential oil of *A. millefolium* phytochemical characterization and its quality.

Ethical approval

Not applicable because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: BD; Design: BD; Supervision: BD; Materials: BD, GÖ; Data Collection and/or Processing: BD, GÖ, DK; Analysis and/or Interpretation: BD, GÖ, DK; Literature Search: GÖ; Writing: BD, GÖ; Critical Reviews: BD.

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Conflict of interest

The authors declared that there is no conflict of interest.

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