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Comparative Chemical Composition and Antimicrobial Activities of The Essential Oils and Solvent Extracts of The Flower, Leaf, And Stem of *Epilobium angustifolium* Growing in Türkiye

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ABSTRACT

Volatile organic compounds (VOCs) of essential oils (EOs) and solid phase microextract (SPME) obtained from the flower, leaf, and stem of *Epilobium angustifolium* L. were analyzed by GC-FID/MS. The EOs and SPMEs consist mainly of monoterpenes and aldehydes, which are major classes of compounds. Limonene was found to be a major compound in flower (HD: 42.9% vs. SPME: 95.5%), in leaf (HD: 60.3% vs. SPME: 4.7%), and in stem (HD: 49.06% vs. SPME: 93.6%). The antimicrobial activity of EOs and the solvent extracts (*n*-hexane, acetonitrile, and methanol) of *E. angustifolium* were screened *in vitro* against nine microorganisms. The EO of the leaf showed the best activity (10.2 µg/mL MIC) against *Mycobacterium smegmatis*. All the EOs and the solvent extracts gave moderate activity against the *Staphylococcus aureus, Bacillus cereus,* and *M. smegmatis* within the range of 10.2-1300.0 µg/mL MIC values. The best antibacterial activity was observed against *S. aureus* and *B. cereus* in the *n*-hexane extract of stem and methanol extract of flower samples.

Keywords: *Epilobium angustifolium*, Essential oil, SPME, GC-FID/MS, Antimicrobial.

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Introduction

Among the known medicinal plant species is *Epilobium* taxa, a worldwide traditional medicinal herb known as fireweed or rosebay willowherb. It is known that the extracts obtained from this plant exhibit various pharmacological effects in traditional medicine [1]. From the past to the present, the *Epilobium* plant's transition from traditional to pharmacological use has clarified the plant's components and biological activities [1,2]. Certain members are commonly utilized in traditional medicine, primarily for gastrointestinal and prostate issues in Türkiye [3] and other countries [4,5]. In traditional use, *Epilobium* taxa are used as a tea to treat headaches, insomnia, infection, anemia, and colds. It has been reported that *Epilobium angustifolium* L. extracts are used for therapeutic purposes in gastrointestinal diseases such as diarrhea, dysentery, ulcers, and urinary diseases such as prostate [1,2,6]. *E. angustifolium* extracts have been shown in investigations to be antiproliferative [7], antiaging [8], antioxidants [9, 10], anti-inflammatory [11], and antibacterial [12-15]. *Epilobium* is a genus of around 185 herbaceous perennial species from the Onagraceae family [1]. *E. angustifolium* (also known as *Chamaenerion angustifolium* (L.) Holub) is native to the temperate Northern Hemisphere [16, 17].

Previous pharmacognostic studies have shown that essential oil from the *E. angustifolium* plant grown in China was obtained using the supercritical CO_2 method, and GC-MS analysis was reported [13]. Headspace-SPME GC-MS was used to investigate the volatile contents of dried and fresh *E. angustifolium*, which grows in Lithuania [18, 19]. Chemical composition, antioxidant, antifungal properties, and skin penetration of *E. angustifolium* grown in Poland were reported. The major components of *E. angustifolium* essential oil were mainly terpenes such as β-linalool, camphor, β-caryophyllene, α-caryophyllene oxide, eucalyptol, and (S)-carvone [20]. Furthermore, there have been a few publications on the phytochemical research done on *E. angustifolium*; these reports mostly focus on the investigation of flavonoids, triterpenoids, polyphenols, and phenolic compounds, and they indicate that these compounds have both antibacterial and immunomodulatory properties [13]. The anticancer properties of *C. angustifolium* were also reported [5, 7, 21-23], and activity was attributed to the oenothein B [24].

Currently, no study has focused on the volatile contents (EOs) and antimicrobial effects for the flower, leaf, and stem of *E. angustifolium* growing in Türkiye. This study explores the antimicrobial activity and chemical

variety of EOs and solvent extracts (*n*-hexane, acetonitrile, and methanol) from the stem, leaf, and flower of *E. angustifolium*. This is the first comparative analysis of the phytochemical content of *E. angustifolium* based on a literature survey. Such a study demonstrates how plant parts alter volatile components and antimicrobial activities.

Materials and Methods

Chemical and Reagents

All solvents (*n*-hexane, acetonitrile, methanol, and dimethyl sulfoxide) and other chemicals (Na_2SO_4, Dabouraud dextrose agar, ampicillin, streptomycin, and fluconazole) used were purchased from Sigma-Aldrich in analytical grade.

Plant Material

Wild-grown *E. angustifolium* was collected from Erzurum (SE part of Türkiye) in August 2022 at an altitude of 1960 m. The fresh plant materials were separated into flower, leaf, and stem parts and air-dried in the shade at room temperature. The plant was identified according to the Flora of Türkiye by Professor Dr. Serdar Makbul [2]. The voucher specimen (Okur 38 & S. Makbul) was deposited in the Herbarium of Recep Tayyip Erdogan University, Department of Biology (RUB), Rize, Türkiye.

Hydrodistillation Apparatus and Procedure

The aerial parts (flowers, leaves, and stems) of *E. angustifolium* (~70 g, fresh) were ground with a mill into small pieces. Grounded parts of the plants were hydrodistillated (HD) with a modified Clevenger-type apparatus (7 °C using Chiller, 3 h), yield (v/w): 0.010%, 0.012%, and 0.011%, respectively. The obtained EOs were extracted using *n*-hexane (0.5 mL, HPLC grade), dried on anhydrous Na_2SO_4, and kept in brown glass bottles in the refrigerator at -10 °C until use [24, 25].

The Solvent Extract of the Flower, Leaf, and Stem of E. Angustifolium

The dried and ground parts of *E. angustifolium* were placed into nine separate 50 mL flasks: flower (8.80 g, 8.92 g, and 8.18 g), leaf (8.62 g, 8.66 g, and 9.20 g), and stem (8.55 g, 8.58 g, and 9.36 g). The materials were extracted three times using analytical grade *n*-hexane, acetonitrile, and methanol (10 mL x 3; 12 h, each). Following solvent filtration, the same extracts were combined and evaporated at 40 °C to yield crude *n*-hexane, acetonitrile, and methanol extracts (0.037 g/0.039 mg/11.3 g), leaf (0.044 g/0.028 g/0.106 g), and stem (0.025 g/0.024 g/0.089 g), respectively.

SPME Analysis

The dry aerial part (flower, leaf, and stem) of *E. angustifolium* (~1.1 g each) was placed in a sealed SPME vial (10 mL) with a silicone-rubber septum cap and exposed to an SPME system (Supelco, USA). A DVB/Carboxen/PDMS coated fiber was utilized to get volatile components. The SPME fibers were heated for conditioning in the GC injector for 5 minutes at 250 °C. Extraction was accomplished utilizing magnetic stirring at 80°C for 5 minutes of incubation and 10 minutes of extraction. After that, fiber containing a volatile chemical extract was injected into the GC injector. A Shimadzu QP2010 Ultra mass selective detector connected to the 2010 Plus chromatograph was used for GC-FID/MS analysis. Helium was the carrier gas with 1 mL/min flow rate. The injection was done in split mode (1:30) at 230 °C. The sample was reported after analysis. The temperature, incubation period, and extraction time were determined based on the previous experiment described [26-28].

GC-FID/ MS Analysis

A Shimadzu QP2010 ultra-GC-FID/MS (Shimadzu Class-5000 Chromatography Workstation software) was used to analyze the EOs. Restek Rxi-5MS capillary column (30 mm x 0.25 mm × 0.25 µm) was used. Injection of the EOs into GC-FID/MS was done in split mode (1:30) at 230°C. The essential oil solution (1 µL) in *n*-hexane (HPLC grade) was injected and analyzed. The column was kept at 60ºC for 2 minutes and then elevated to 240ºC using a 3ºC/min heating ramp. The oven program was as follows: the initial temperature was 60°C for 2 minutes, then rose to 240°C after 3 minutes, and finally held at 250°C for 4 minutes. The carrier gas utilized was helium (99.999%) at a steady flow rate of 1 mL/min. The ionization voltage was set at 70 eV, the detection was carried out in electronic impact mode (EI), and mass acquisition was done using scan mode (40-450 m/z). Each sample was evaluated, and the average was given [26, 27]. The volatile components were identified by comparing the literature RI and library mass values (NIST, Wiley7NL, FFNSC1.2, and W9N11) [25-34].

Antimicrobial Activity

All test microorganisms were obtained from the Hıfzısihha Institute of Refik Saydam (Ankara, Türkiye). They were *Bacillus cereus* 709 ROMA; Candida *albicans* ATCC 60193, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Mycobacterium smegmatis* ATCC607, *Pseudomonas aeruginosa* ATCC 27853, *Saccharomyces cerevisiae* RSKK 251, *Staphylococcus aureus* ATCC 25923, and *Yersinia pseudotuberculosis* ATCC 911. The plant solvent extracts were dissolved in *n*-hexane, acetonitrile, and methanol to prepare an extract stock solution. Antimicrobial susceptibility of the EOs, *n*-hexanes, acetonitrile, and methanol extracts of *E. angustifolium* were screened using the agar-well diffusion method [27, 35, 36]. Each bacterium and yeast were cultured in Mueller Hinton (MH) (Difco, Detroit, MI) broth and yeast extract broth, respectively. Then, the microorganisms were diluted with approximately 10^6 colony-forming units (CFU) per mL. Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) was used for yeast-like fungi. Microorganisms were "flood-inoculated" onto MH and SD agars and dried under aseptic conditions. 50 µL of essential oils and solvent extracts of *E. angustifolium* were

delivered into wells (5 mm diameter), opened on agar plates, and incubated at 35 °C for 18h. The *M. smegmatis* was grown for 3 to 5 days on MHA plates at 35 °C. Microbial activity was evaluated by measuring the inhibition zone diameters. Antimicrobial agents such as Ampicillin (10 µg/mL), streptomycin (10 µg/mL), and fluconazole (5 µg/mL) were used as the positive control.

Agar Dilution MIC Assay

The antimicrobial assay of EOs, *n*-hexane, acetonitrile, and methanol extracts of *E. angustifolium* was worked quantitatively in respective broth media using double microdilution method. The antibacterial and antifungal assays were carried out in Mueller-Hinton broth (MH) at pH. 7.3 and buffered Yeast Nitrogen Base at pH 7.0, respectively. The microdilution test plates were incubated at 35°C for 18h [27-29]. Brain Heart Infusion broth was used for *M. smegmatis* and incubated at 35°C for 48-72h. The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10 µg/mL), streptomycin (10 µg/mL), and fluconazole (5 µg/mL) were used as standard antibacterial and antifungal drugs. Dimethyl sulfoxide with a dilution (1:10) was used as solvent control.

Results and Discussion

Chemical Constituents of EOs and SPME

A total of 34/15, 45/32, and 39/21 constituents were discovered in the flower, leaf, and stem of *E. angustifolium*, accounting for 91.8-98.8% in HD and 92.3-99.7% in SPME, according to GC-FID/MS analysis for the flower, leaf, and stem of *E. angustifolium*, respectively. The volatile organic components of EOs and SPME in the flower, leaf, and stem of *E. angustifolium*, as well as their KI and percentages, are listed in Table 1.

The EOs were characterized by comparing mass spectra in the libraries (NIST, Wiley7NL, FFNSC1.2, and W9N11) and utilizing the Kovats index [27, 33]. The EOs have been analyzed on the Rxi-5MS column [24-26]. Eighty-one volatile compounds were detected in the EOs and SPME of *E. angustifolium* by the GC-FID/MS analysis. Results had shown that EOs had higher chemical diversity than SPMEs (Table 1). Significant quantitative and qualitative differences were observed between EOs and SPME and among the flower, leaf, and stem samples (Table 1). A variety of chemical classes, including aliphatic hydrocarbons, aldehydes, alcohols, esters, acids, and other hydrocarbons, as well as monoterpenes, sesquiterpenes, and terpene-related substances, are among the components that have been found. The EOs and SPMEs consisted mainly of monoterpenes (flower, HD: 50.9% vs. SPME: 98.1%; leaf, HD: 68.6% vs. SPME: 72.3%; stem, HD: 57.5% vs. SPME: 96.1%), and aldehydes (flower, HD: 7.9% vs. SPME: 0.9%, leaf, HD: 6.5% vs. SPME: 6.2%, stem, HD: 4.7% vs. SPME: 1.3%). Limonene (flower, HD: 42.9% vs. SPME: 95.5%, leaf, HD: 60.3% vs. SPME: 4.7%, stem, HD: 49.06% vs. SPME: 93.6%) was a major compound of the EOs and SPMEs of *E. angustifolium.* Sesquiterpenoids were the minor constituents for only EOs in all parts (flower, HD: 1.6%, leaf, HD: 0.2%, stem, HD: 0.1%) of the *E. angustifolium.* The quantitative alterations in compounds and the appearance/disappearance of volatiles were the two effects of *E. angustifolium* extractions on the plant's composition. Specific molecules, such as (*Z*)-3-hexenol, 2-heptanol, tetradecane, methyl palmitate, *n*-hexadecanoic acid, ethyl palmitate, methyl linolenate, and phytol were found only in EOs samples of *E. angustifolium*. At the same time, (*E*)-2-hexenal, *β*-ocimene, and *β*-cadinene existed only in the SPME samples of *E. angustifolium.* Out of 81 chemicals, only five were found in all samples, including *n*-hexanal (0.5%, 0.1%, 0.5%, 0.2%, 0.7%, and 0.2%), *α*-pinene (1.5%, 0.31%, 1.5%, 2.7%, 1.1%, and 0.4%), sabinene (0.2%, 0.3%, 0.6%, 2.0%, 0.4%, and 0.3%), *β*-mycrene (1.7%, 1.7%, 2.0%, 1.3%, 1.6%, and 1.8%), and limonene (42.9%, 95.51%, 60.3%, 4.7%, 49.0%, and 93.6%), respectively. The quantitative makeup of the volatiles changed depending on where the sample came from. For example, the percentage content of limonene in EO samples varied from 42.9% to 60.3%, and in SPME samples were in the range of 4.7% and 95.5% (Table 1). The variety in chemical components, as reported in the literature [24-30], could be attributed to differences in plant parts and extraction procedures-consequently, the flower, leaf, and stem of *E. angustifolium* growing in Türkiye exhibit distinct chemotypes. A comparative investigation of the plant's sections revealed that monoterpenes (flower, 98.1%, leaf, 72.3%, and stem, 96.1%) were the major components of *E. angustifolium* SPMEs. Some of the identified compounds may form by auto-oxidation during the HD. In our study, eugenol was seen only in the flower essential oil isolated by HD. The numbers of terpenes/terpenoids identified from *E. angustifolium* using HD and SPME were different (flower, HD: 11 vs. SPME: 10; leaf, HD: 17 vs. SPME: 22; stem, HD: 18 vs. SPME: 13).

In the literature, the EO of *E. angustifolium* was obtained by supercritical carbon dioxide. Linoleic acid (17.1%), 1-docosene (11.4%), hexadecanoic acid (10.6%), and linolenic acid (7.3%) were reported to be major constituents, and only 13 out of 78 compounds were the same as in our case [13]. EO also inhibited *E. coli*, *Bacillus subtilis*, *Micrococcus luteus*, and *Enterobacter aerogenes* [13].The essential oil for the dried herbs of *E. angustifolium* from Poland yielded 24 compounds and gave the highest concentration of cosanes (23.70%), 5-methyl docosane (14.95%), and *α*-caryophyllene oxide (8.57%) [20].The volatile constituents of fresh and air-dried samples of *C. angustifolium* were analyzed using HS-SPME GC-MS. Trans-2-hexenal (16.0-55.9% of all volatiles) and trans-anethole (2.6-46.2%) were present only in the dried samples, while *cis*-3-hexenol (17.5-68.6 %) was only in fresh samples and 11 out of 42 constituents were the same as in our result [18]. α/β-Caryophyllenes were reported in all analyzed samples, contributing from 2.4% to 52.3% (fresh or dried).

Table 1. Volatile organic compounds for the flower, leaf, and stem of *E. angustifolium*

No	Compounds	RI*	RI[a]	(%)[b]					
				Flower		Leaf		Stem	
				HD	SPME	HD	SPME	HD	SPME
1.	2-Ethylfuran	728	733	-	-	-	0.1	-	-
2.	Methylbenzene	782	789	-	-	-	0.1	-	-
3.	Hexanal	803	802	0.5	0.1	0.5	0.2	0.7	0.2
4.	(*E*)-2-Hexenal	852	848	-	0.2	-	5.7	-	0.2
5.	(*Z*)-3-Hexenol	858	859	1.6	-	3.0	-	1.3	-
6.	2-Heptanol	894	891	2.1	-	1.2	-	0.6	-
7.	2,4-Hexadienal	907	904	-	-	-	0.2	-	-
8.	*α*-Thujene	931	930	-	-	-	1.4	-	-
9.	*α*-Pinene	940	938	1.5	0.3	1.5	2.7	1.1	0.4
10.	Benzaldeyhde	960	963	4.2	-	2.4	-	2.4	-
11.	Sabinene	978	976	0.2	0.3	0.6	2.0	0.4	0.3
12.	*β*-Pinene	980	981	1.6	-	2.0	9.3	1.8	-
13.	*β*-Mycrene	992	989	1.7	1.7	2.0	1.3	1.6	1.8
14.	Octanal	1003	1001	-	0.4	1.1	-	-	0.2
15.	*α*-Phellandrene	1002	1006	-	-	-	0.1	-	-
16.	*α*-Terpinene	1018	1018	-	-	-	0.5	-	-
17.	*o*-Cymene	1022	1027	0.4	-	0.1	15.1	0.4	-
18.	Limonene	1031	1035	42.9	95.5	60.3	4.7	49.0	93.6
19.	Benzene acetaldeyhde	1052	1055	2.9	-	1.2	-	1.0	-
20.	*β*-Ocimene	1046	1045	-	0.3	-	5.8	-	0.1
21.	*γ*-Terpinene	1060	1059	2.6	-	2.1	29.4	2.8	-
22.	Octanol	1063	1068	-	-	0.7	-	-	-
23.	Linalool oxide	1073	1073	-	-	0.6	-	-	-
24.	*α*-Terpinolene	1191	1089	-	-	-	-	0.4	-
25.	2-Nonanol	1097	1097	4.3	-	-	-	-	-
26.	2-Ethyl-*p*-xylene	1085	1089	-	-	-	7.4	-	-
27.	Linolool	1098	1096	-	0.1	5.5	0.7	6.0	0.1
28.	Nonanal	1101	1101	0.3	-	0.1	-	-	-
29.	Citronella	1148	1151	-	-	0.1	-	-	-
30.	(*Z*)-2-Nonenal	1142	1142	-	-	-	-	0.1	0.6
31.	4-Terpineol	1192	1190	0.8	-	1.1	-	-	-
32.	*α*-Terpineol	1191	1189	0.3	-	0.9	0.1	0.3	0.9
33.	Decanal	1201	1201	-	0.2	0.8	0.1	0.4	0.1
34.	*β*-Cyclocitral	1220	1223	-	-	0.4	-	-	-
35.	Geraniol	1240	1249	-	-	-	-	-	0.1
36.	Neral	1235	1240	-	-	0.2	-	-	-
37.	Geranial	1264	1268	-	-	-	-	-	0.1
38.	Tridecane	1300	1297	2.4	-	1.3	0.1	2.9	-
39.	Undecanal	1305	1302	-	-	0.1	-	-	-
40.	Bicyloelemene	1333	1331	-	-	-	-	-	0.1
41.	Eugenol	1356	1358	1.9	-	-	-	-	-
42.	Decanoic acid	1364	1364	-	-	0.3	-	-	-
43.	*β*-Cubenene	1387	1390	-	-	-	0.1	-	-
44.	*α*-Copaene	1374	1370	-	0.1	0.3	-	-	0.1
45.	*β*-Damascenone	1383	1387	-	-	0.3	-	0.3	-
46.	Tetradecane	1400	1403	2.4	-	1.0	-	2.8	-
47.	*β*-Elemene	1389	1394	-	-	-	6.1	-	-
48.	*α*-Bergamotene	1411	1414	-	-	-	-	-	0.2
49.	Dodecanal	1408	1409	-	-	0.1	-	0.1	-
50.	*β*-Caryophyllene	1417	1416	-	0.2	0.9	2.7	-	0.1
51.	*β*-Copaene	1430	1430	-	-	-	0.1	-	-
52.	Geranyl acetone	1145	1444	-	-	-	-	0.3	
53.	Neryl acetone	1435	1439	-	-	0.1	-	-	-
54.	*α*-Humulene	1460	1460	-	-	0.3	0.6	-	-
55.	Valencene	1496	1495	-	0.2	-	-	0.2	0.1
56.	D-Germacrene	1484	1487	-	-	-	0.1	-	-
57.	*β*-Ionone	1487	1487	0.2	-	0.7	-	0.3	0.3
58.	*β*-Selinene	1493	1493	-	-	-	0.1	-	-
59.	*α*-Farnesene	1505	1504	-	-	-	2.1	-	-
60.	Methyl laurate	1526	1528	-	-	-	-	0.1	-
61.	*β*-Cadinene	1518	1516	-	0.1	-	0.1	-	0.1
62.	*α*-Cadinene	1526	1537	-	-	-	-	0.3	-
63.	Dodecanoic acid	1526	1528	-	-	-	-	0.1	-
64.	*α*-Cadinol	1652	1651	-	-	0.1	-	0.1	-
65.	*α*-Bisabolol	1685	1686	1.6	-	0.2	-	-	-
66.	Methyl myristate	1726	1727	0.1	-	0.1	-	-	-
67.	Tetradecanoic acid	1763	1765	0.1	-	-	-	0.1	-
68.	Ethyl myristate	1790	1786	0.2	-	0.1	-	-	-

69.	Hexahydrofarnesyl acetone	1847	1848	-	-	0.1	-	0.1	-
70.	Nonadecane	1900	1900	0.3					
71.	Methyl palmitate	1926	1928	0.1	-	0.4	-	1.0	-
72.	*n*-Hexadecanoic acid	1966	1968	3.4	-	0.3	-	5.6	-
73.	Ethyl palmitate	1990	1986	3.7	-	0.5	-	1.7	-
74.	Heneicosane	2100	2098	6.3	-	-	0.1	-	-
75.	Methyl linolenate	2101	2097	5.4	-	1.7	-	5.2	-
76.	Phytol	2110	2110	0.5	-	0.3	-	0.2	-
77.	Linoleic acid	2131	2132	0.4	-	-	-	1.2	-
78.	Oleic acid	2152	2156	-	-	0.4	0.4	3.5	-
79.	Ethyl stearate	2194	2195	0.1	-	-	-	-	-
80.	Docosane	2200	2199	-	-	-	-	-	-
81.	Tricosane	2300	2298	1.8	-	0.2	0.2	0.8	-
	Chemical classes			**Area% [b] and NC[c]**					
	Monoterpene hydrocarbons			50.9:7	98.1:5	68.6:7	72.3:11	57.5:8	96.2:5
	Monoterpenoids			3.0:3	0.1:4	8.8:7	0.8:2	0.9:2	1.2:4
	Sesquiterpene hydrocarbons			-	0.6:1	1.5:3	12.0:9	0.5:2	0.7:6
	Sesquiterpenoids			1.6:1	-	0.2:2	-	0.1:1	-
	Terpene related to compounds			0.2:1	-	1.2:4	-	1.0:4	0.3:1
	Aliphatic hydrocarbons			13.2:5	-	2.5:3	0.4:3	6.5:3	-
	Aldehydes			7.9:4	0.9:4	6.5:8	6.2:4	4.7:6	1.3:5
	Alcohols			8.0:3	-	4.9:3	-	1.9:2	-
	Esters			9.6:6	-	2.8:5	-	8.0:5	-
	Acids			3.9:3	-	1.0:3	0.4:1	10.5:5	-
	Others			0.5:1	-	0.3:1	0.2:2	0.2:1	-
	Total			98.8:34	99.7:15	98.3:45	92.3:32	91.8:39	99.7:21

* Retention Index of references; [a] Retention Index calculated from retention times relative to that of *n*-alkane (C_6-C_{32}) series; [b] Percentages obtained by FID peak-area normalization; [c] NC: Number of compounds.

The ethanolic extract's total phenolic compounds and the DPPH free radical scavenging activity (in fresh sample 238.6-557.1 mg/g) were also reported [18]. In another work, 42 aliphatic hydrocarbons, aldehyde, and alcohols were mentioned from the *t*-butyl methyl ether extract of *C. angustifolium* leaf and stem [21]. Total phenolic contents, antioxidant activity, and volatile constituents of *C. angustifolium,* collected in six different locations in Lithuania, were reported using SPME GC-MS. Volatile analysis of all samples had been classified into two significant chemotypes, such as *α*-/*β*-caryophyllenes and anethole [19]. Alcohol for the EO and SPME and aromatic hydrocarbons for the SPME of the *n*-hexane extract of *Epilobium hirsutum* L. were reported to be major classes of constituents [37].

As previously stated, the cost of extraction is advantageous for the SPME approach in terms of time and decomposition. The chemical variations in the VOCs on *Epilobium* taxa may be due to environmental and analysis conditions. This work showed that the extraction methods used had remarkable changes in volatile constituents. The literature also demonstrated the differences in volatile composition [30, 34]. However, using different extraction methods allows the appearance of new compounds to have a positive effect on the quality of *E. angustifolium*. Limonene (95.5% and 93.6%) was highly content determined from the SPMEs of the flower and stem, respectively. It was found that *E. angustifolium* oil rich in monoterpene in the range of 50.9% to 98.1% which showed significant inhibitory activity against *C. albicans, Candida glabrata*, *Aspergillus nigerand*, *Bacillus subtilis,* and *P. aeruginosa*. However, no activity was reported against the *Y. pseudotuberculosis*. The evaluation of antimicrobial activities for the pure compounds of *E. angustifolium* was beyond the scope of this work. However, according to the above-mentioned published data [6-19], EOs and solvent extracts of this herb can also be of therapeutic value.

The amounts of monoterpenes found by SPME were greater in HD volatiles for the flower and stem, such as limonene (flower, HD: 42.9% vs. SPME: 95.58%, and stem, HD: 49.0% vs. SPME: 93.6%). This is probably related to the volatility because of a shorter extraction time (10 min for SPME vs. 3 h for HD). Thus, limonene could be used as a taxonomical marker for classifying *E. angustifolium*.

Antimicrobial Activities

The antimicrobial activity of EOs and solvent extracts (*n*-hexane, acetonitrile, and methanol) of the flower, leaf, and stem of *E. angustifolium* was tested using the agar well diffusion method against *E. coli*, *Y. pseudotuberculosis*, *Y. coli*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *B. cereus*, *M. smegmatis*, *C. albicans*, and *S. cerevisiae* (Table 2) [35, 36]. In general, EOs and *n*-hexane extract showed moderate antimicrobial activities against *S. aureus, B. cereus, M. smegmatis*, *C. albicans*, and *S. cerevisiae* with the inhibition 6-27 mm range, respectively (Table 2).

Table 2. Antimicrobial assay of EOs and solvent extracts for the flower, leaf, and stem of *E. angustifolium*

Sample Extracts	Plant parts	Const. (µg/ml)		Microorganisms, inhibition zone (mm), and MIC (µg/mL)								
				Gram (-)			Gram (+)			No Gr.	Fungi	
				Ec	*Yp*	*Pa*	*Ef*	*Sa*	*Bc*	*Ms*	*Ca*	*Sc*
EOs	Flower	17900	mm	-	-	-	-	6	7	15	-	-
			MIC	-	-	-	-	895	895	223.8	-	-
	Leaf	26000	mm	-	-	-	-	6	9	27	7	10
			MIC	-	-	-	-	1300	1300	10.2	1300	650
	Stem	16900	mm	-	-	-	-	6	6	18	-	-
			MIC	-	-	-	-	845	845	105.6	-	-
n-Hexane	Flower	19000	mm	-	-	-	-	14	14	15	12	15
			MIC	-	-	-	-	118.8	118.8	59.4	118.8	59.4
	Leaf	17000	mm	-	-	-	-	8	8	16	16	20
			MIC	-	-	-	-	425	425	53.1	53.1	13,3
	Stem	12500	mm	6	-	-	-	12	14	15	16	20
			MIC	625	-	-	-	78.1	39.1	39.1	19.5	9.7
CH_3CN	Flower	29300	mm	6	-	12	18	15	15	15	-	8
			MIC	1465	-	732.5	366.3	366.3	366.3	91.6	-	1465
	Leaf	16400	mm	6	-	13	14	10	14	15	-	-
			MIC	820	-	410	410	410	410	51.3	-	-
	Stem	10300	mm	-	-	-	-	10	11	8	-	6
			MIC	-	-	-	-	515	515	515.0	-	515
CH_3OH	Flower	61100	mm	6	-	15	10	14	15	12	-	-
			MIC	1527	-	190	381.9	95.5	95.5	95.5	-	-
	Leaf	60000	mm	-	-	10	10	8	10	15	-	-
			MIC	-	-	750	750	750	750	93.8	-	-
	Stem	44700	mm	-	-	10	8	17	14	12	-	-
			MIC	-	-	558.8	1117	69.9	69.9	69.9	-	-
Amp.		10		10	10	18	10	35	15			
Strep.		10								35		
Flu.		5									25	25

Ec: E. coli, *Yp*: *Y. pseudotuberculosis*, *Pa*: *P. aeruginosa*, *Sa*: *S. aureus*, Ef: *E. faecalis*, Bc: *B. cereus*, Ms: *M. smegmatis*, *Ca*: *C. albicans*, *Sc*: *S. cerevisiae*, Amp.: Ampicillin, Strep.: Streptomycin, Flu.: Fluconazole, (-): no activity of test concentrations.

The best activity was observed for the EO of the leaf against *M. smegmatis* with 27 mm inhibition. Acetonitrile flower extract gave better activity against the *P. aeruginosa, E. faecalis, S. aureus, B. cereus*, and *M. smegmatis* with 12 mm, 18 mm, 15 mm, 15 mm, and 15 mm inhibition zones, respectively. The methanol extract showed moderate antimicrobial activity against all tested microorganisms except *Y. pseudotuberculosis, C. albicans*, and *S. cerevisiae*. The results have shown that the EOs and extracts' antimicrobial activity are more susceptible to gram-(+) bacteria and *M. smegmatis*. The MIC of the EOs and the extracts from the parts of *E. angustifolium* were observed within the range of 10.2-1465.0 µg/mL against *E. coli, P. aeruginosa, E. faecalis, S. aureus, B. cereus,* and *M. smegmatis,* respectively (Table 2). Limonene (varied from 42.9% to 95.5%) was the major compound in the EOs and SPME of all, except *γ*-terpinene (29.4%) was the main constituent in the SPME of the leaf, which has been reported to have antibacterial properties [38]. Thus, the bactericidal activity of the EOs and solvent extracts of *E. angustifolium* may be mainly related to the high content of monoterpenes. Other constituents present in the extracts were reported to have antibacterial activities and may also have made a remarkable contribution to the bactericidal activities of EOs and solvent extracts. The antibacterial activity differences are possibly due to the composition, the concentration of EOs, and extraction methods.

A phytochemical study (six flavonoids and 4 phytosterols) and antimicrobial activity of the aqueous and ethanolic extracts of *E. angustifolium* were reported [16]. 92 Neutral compounds (triterpenoids, polyprenols, and dolichols) were mentioned from *C. angustifolium* leaves [21]. Antibacterial, antifungal, antioxidant activities, total phenolic content, DNA-binding activity for the methanolic, ethanolic, and aqueous extracts of *E. angustifolium* were mentioned [6]. Ethanol extracts of *E. angustifolium* leaf and flower tested against the of *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *Proteus mirabilis*, *C. albicans*, *C. tropicalis*, *C. dubliniensis* and *S. cerevisiae* with MIC values between 4.6±0.2 and 18.2±0.8 mg/mL [14]. Total flavonoid content, antioxidant activity, elastase, tyrosinase, and lipoxygenase inhibitor capacity of the aqueous extract of *E. angustifolium* were reported. The aqueous extracts of *E. angustifolium* exhibited strong elastase (EC_{50} = 42.72

± 2.38 µg/mL), tyrosinase (EC_{50} = 33.03 ± 3.71 µg/mL), and lipoxygenase inhibitory activities (EC_{50} = 0.57 ± 0.06 µg /mL) [12]. The antimicrobial activity for the ethanolic extracts of *Epilobium* species (*E. angustifolium*, *E. hirsutum*, *E. palustre*, *E. tetragonum,* and *E. rosmarinifolium*) was mentioned, and all the ethanolic dry extracts gave antimicrobial activity in a range of 10 µg/mL to 650 µg/mL [16]. The antimicrobial activities of the EO of *E. hirsutism* have been reported, and it showed activity against *E. coli* with an inhibition zone of 10 mm [37].

Conclusion

Eighty-one volatile constituents were characterized from the flower, leaf, and stem of *E. angustifolium,* resulting in variations containing different VOCs. The amounts of monoterpenes identified by SPME of *E. angustifolium* were more significant in EOs for the flower and stem of *E. angustifolium.* Limonene (varied from 42.9% to 95.5%) was the major compound in the EOs and SPME of all, whereas *γ*-terpinene (29.4%) was the main constituent in the SPME of the leaf. Experimental results showed that different extraction methods and parts of the plant used gave chemical variation as in the literature. The amount of limonene was so high that it could be the source to produce it. All EOs and the solvent extracts gave good activity against the *M. smegmatis* within the range of 10.2-223.8 µg/mL (MIC). In general, the leaf's most significant activity of EO was observed at 10.2 µg/mL MIC value against *M. smegmatis*. *n*-Hexane of leaf extract gave better antimicrobial activity against the *S. aureus, B. cereus,* and *M. smegmatis* with 39.1 µg/mL, 39.1 µg/mL, and 78.1 µg/mL MIC values, respectively. *n*-Hexane extracts of the leaf and stem of the plant gave good activity against yeast fungi (antifungal activity). According to MIC values, the highest antimicrobial activity was observed against gram (+) bacteria and *M. smegmatis*. Limonene was used as an aroma to mask the bitter taste of alkaloids in pharmaceutical production. It was used industrially in food production, perfumery, and personal care products such as shaving lotions. Therefore, the overall antimicrobial activity results suggested that EOs and extracts of *E. angustifolium* may have promising prospects for industrial or pharmaceutical applications. In future work, activity-guided phytochemical studies could be carried out for the solvent extract of *E. angustifolium.*

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Conflict of Interest

No potential conflict of interest was reported by the author(s).

References

[1] Schepetkin I.A., Ramstead A.G., Kirpotina L.N., Voyich J.M., Jutila M.A., Quinn M.T., Therapeutic potential of polyphenols from *Epilobium angustifolium* (Fireweed), Phytother. Res., 30(8) (2016) 1287-1297.

[2] Lans C., Possible similarities between the folk medicine historically used by First Nations and American Indians in North America and the ethnoveterinary knowledge currently used in British Columbia, Canada. J. Ethnopharmacol., 192 (2016) 53-66.

[3] Zeybek N., Zeybek U., Pharmaceutical botany. Ege University Faculty of Pharmacy Publication, Izmir (1994).

[4] Granica S., Piwowarski J.P., Czerwińska M.E., Kiss A.K., Phytochemistry, pharmacology and traditional uses of different *Epilobium* species (Onagraceae): A review, J. Ethnopharmacol., 156 (2014) 316-346.

[5] Sayik A., Yusufoğlu A.S., Leyla A., Türker G., Aydin B., Arslan L., DNA-Binding, Biological Activities and Chemical Composition of Wild Growing *Epilobium* angustifolium L. Extracts from Çanakkale, Turkey, J. Turk. Chem. Society, 4(3) (2017) 811-840.

[6] Vitalone A., Guizzetti M., Costa L.G., Tita B., Extracts of various species of *Epilobium* inhibit proliferation of human prostate cells. J. Pharm. Pharmacol., 55(5) (2003) 683-690.

[7] Vitalone A., Bordi F., Baldazzi C., Mazzanti G., Saso L., B. Tita, Anti-proliferative effect on a prostatic epithelial cell line (PZ-HPV-7) by *Epilobium angustifolium* L., Farmaco. 56 (5-7) (2001) 483-489.

[8] Ruszová E., Cheel J., Pávek S., Moravcová M., Hermannová M., Matějková I., Spilková J., Velebný V., Kubala L., *Epilobium angustifolium* extract demonstrates multiple effects on dermal fibroblasts in vitro and skin photo-protection *in vivo*, Gen. Physiol. Biophys., 32(3) (2013) 347-359.

[9] Tóth B.H., Blazics B., Kéry Á., Polyphenol composition and antioxidant capacity of *Epilobium* species. J. Pharm. Biomed. Anal., 49(1) (2009) 26-31.

[10] Onar H.C., Yusufoglu A., Turker G., Yanardağ R., Elastase, tyrosinase and lipoxygenase inhibition and antioxidant activity of an aqueous extract from *Epilobium angustifolium* L. leaves, J. Med. Plant Res., 6(5) (2012) 716-726.

[11] Juan H., Sametz W., Hiermann A., Anti-inflammatory effects of a substance extracted from *Epilobium angustifolium,* Agents and Actions, 23(1-2) (1988) 106-107.

[12] Kosalec I., Kopjar N., Kremer D., Antimicrobial activity of Willowherb *(Epilobium angustifolium L.)* leaves and flowers, Curr. Drug Targets, 14(9) (2013) 986-991.

[13] Zeng Q., Wu J., Lin P., Chemical composition and antimicrobial activity of the essential oil from *Epilobium angustifolium*, Chem. Nat. Compd., 52(6) (2016) 1113-1115.

[14] Battinelli L., Tita B., Evandri M.G., Mazzanti G., Antimicrobial activity of *Epilobium spp*. Extracts, Farmaco., 56(5-7) (2001) 345-348.

[15] Silló S., Varga E., Belák Á., Maráz A., Phytochemical and antimicrobial investigation of *Epilobium angustifolium* L, Acta Pharm. Hung., 84(3) (2014) 105-110.

[16] Baum D.A., Sytsma K.J., Hoch P.C., A phylogenetic analysis of *Epilobium (Onagraceae)* based on nuclear ribosomal DNA sequences, Systematic Botany, 19(3) (1994) 363-388.

[17] Chamberlain D., Raven P.H., Epilobium *L.* Flora of Turkey and The East Aegean Island*.*, Edinburgh University Press (1972) 183-196.

[18] Kaškonienė V., Stankevičius M., Drevinskas T., Akuneca I., Kaškonas P., Survilienė K.B., Maruška A., Ragažinskienė O., Kornyšova O., Briedis V., Ugenskienė R., Evaluation of phytochemical composition of fresh and dried raw material of introduced *Chamerion angustifolium* L. using chromatographic, spectrophotometric and chemometric techniques, Phytochem*.*, 115 (2015) 184-193.

[19] Kaškonienė V., Maruška A., Akuņeca I., Stankevičius M., Ragažinskienė O., Bartkuvienė V., Kornyšova O., Briedis V., Ugenskienė R., Screening of antioxidant activity and volatile compounds composition of *Chamerion angustifolium (L.)* Holub ecotypes were grown in Lithuania, Nat. Prod. Res., 30(12) (2016) 1373-1381.

[20] Nowak A., Duchnik W., Makuch E., Kucharski L., Ossowicz-Rupniewska P., Cybulska K., Sulikowski T., Moritz M., Klimowicz A., *Epilobium angustifolium* L. Essential oil-biological activity and enhancement of the skin penetration of drugs-*in vitro* study, Molecules, 26(23) (2021) 7188.

[21] Kukina T., Frolova T., Salnikova O., Neutral constituents of *Chamaenerion angustifolium* leaves, Chem. Nat. Compd*.,* 50(2) (2014) 233-236.

[22] Kiss A., Kowalski J., Melzig M., Induction of neutral endopeptidase activity in PC-3 cells by an aqueous extract of *Epilobium angustifolium* L. and oenothein B, Phytomedicine, 13(4) (2006) 284-289.

[23] Kiss A.K., Bazylko A., Filipek A., Granica S., Jaszewska E., Kiarszys U., Kośmider A., Piwowarski J., Oenothein B's contribution to the anti-inflammatory and antioxidant activity of *Epilobium* sp., Phytomedicine, 18(7) (2011) 557-560.

[24] Schepetkin I.A., Kirpotina L.N., Jakiw L., Khlebnikov A.I., Blaskovich C.L., Jutila M.A., Quinnet M.T., Immunomodulatory activity of oenothein B isolated from *Epilobium angustifolium*, J. Immunol. Res., 183(10) (2009) 6754-6766.

[25] Tosun G., Kahriman N., Albay C., Karaoğlu Ş.A., Yaylı N., Antimicrobial activity and volatile constituents of the flower, leaf, and stem of *Paeonia daurica* grown in Turkey, Turk. J. Chem., 35(1) (2011) 145-153.

[26] Renda G., Özel A., Barut B., Korkmaz B., Yaylı N., The Volatile Chemical Compositions of the Essential Oil/SPME and Enzyme Inhibitory and Radical Scavenging Activities of Solvent Extracts and the Essential oils from *Coronilla orientalis* Miller and *C. varia* L. grows in Turkey, Iran. J. Pharm. Res., 18(4) (2019) 1831-1842.

[27] Renda G., Celik G., Korkmaz B., Karaoglu S.A., Yaylı N., Antimicrobial activity and analyses of six *Geranium* L. species with headspace SPME and hydrodistillation, J. Essent. Oil-Bear. Plants, 19(8) (2016) 2003-2016.

[28] Yaylı B., Tosun G., Karaköse M., Renda G., Yaylı N., SPME/GC-MS analysis of volatile organic compounds from three *Lamiaceae* species *(Nepeta conferta Hedge & Lamond, Origanum onites* L. and *Satureja cuneifolia Ten*.) growing in Turkey, Asian J. Chem., 26(9) (2014) 2541-2544.

[29] Yaylı N., Yaşar A., Yaylı N., Albay M., Coşkunçelebi K., Essential Oil Analysis and Antimicrobial Activity of *Paeonia mascula* from Turkey, Nat. Prod. Commun., 3(6) (2008) 941-944.

[30] Iskender N.Y., Yaylı N., Yildrim N., Cansu T.B., Terzioglu S., The volatile constituents of the flower, leaf, and stem of *Verbascum wiedemannianum* grown in Turkey, J. Oleo Sci., 58(3) (2009) 117-121.

[31] Yılmaz G., Çiçek M., Demirci B., Başer K.H.C., Composition of the essential oils of five subspecies of *Scutellaria orientalis* from Turkey, J. Essent. Oil Res., 32(5) (2020) 429-435.

[32] Verma R.S., Verma S.K., Tandon S., Padalia R.C., Darokar M.P., Chemical composition and antimicrobial activity of *Java citronella (Cymbopogon winterianus Jowitt ex Bor)* essential oil extracted by different methods, J. Essent. Oil Res., 32(5) (2020) 1-7.

[33] Adams R.P., Identification of essential oil components by gas chromatography/mass spectrometry. Allured publishing corporation Carol Stream, IL (2007).

[34] Üçüncü O., Yaylı N., Yaşar A., Terzioğlu S., Yaylı N., Chemical Composition of the Essential Oils from Flower, Leaf, and Stem of *Senecio trapezuntinus* Boiss. Grown in Turkey, Nat. Prod. Commun., 3(6) (2008) 925-928.

[35] Barry A.L., Standards NCCLS document, Methods for determining bactericidal activity of antimicrobial agents: approved guideline. National Committee for Clinical Laboratory Standards Wayne (1999).

[36] Woods G.L., Brown-Elliott B.A., Conville P.S., Desmond E.P., Hall G.S., Lin G., Pfyffer G.E., Ridderhof J.C., Siddiqi S.H., Wallace R.J., Warren N.G., Witebskyet F.G., Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes. Wayne (PA): Clinical and Laboratory Standards Institute (2011).

[37] Kılıç G., Korkmaz B., Erik İ., Fandaklı S., Yaylı S.S., Faiz Ö., Karaoğlu Ş.A., Yaylı N., Antimicrobial, antioxidant, tyrosinase activities and volatile compounds of the essential oil and solvent extract of *Epilobium hirsutum* L. growing in Turkey, Turkish Journal of Analytical Chemistry, 2(2) (2020) 87-94.

[38] Erasto P., Viljoen A.M., Limonene-A review: Biosynthetic, ecological and pharmacological relevance, Nat. Prod. Commun., 3(7) (2008) 1190-1202.