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Antimicrobial, antioxidant, tyrosinase activities and volatile compounds of the essential oil and solvent extract of *Epilobium hirsutum* L. growing in Turkey

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Abstract

The essential oil (EO), solid phase micro extraction (SPME), and SPME of *n*-hexane extract of *Epilobium hirsutum* L. were analyzed by GC-FID/MS. A total of 35, 20, and 32 compounds were identified from *E. hirsutum*, accounting for 98.2% in hydrodistillation (HD), 97.6% in SPME, and 98.8% in SPME of *n*-hexane extract, respectively. The EO and SPME consisted mainly of alcohol (HD: 51.5% vs. SPME: 30.8%). Aromatic hydrocarbons (57.1%) were the major compounds for the SPME of *n*-hexane extract. Monoterpenes were the minor constituents for the EO (2.3%) and SPME (3.1%) of the *E. hirsutum*. (Z)-3-Hexene-1-ol (46.5%), (Z)-3-hexenyl acetate (18.2%), and cyclohexanone (38%) were major compounds for the EO, SPME, and SPME of *n*-hexane of the *E. hirsutum*, respectively. The comparative study gave that the total amount of monoterpenoids (6.3%) was found only in the EO of *E. hirsutum*. The antimicrobial activities of EO and solvent extracts (*n*-hexane, methanol, and water) of *E. hirsutum* were screened in vitro against nine microorganisms. The EO resulted in the best activity (10 mm) against *Escherichia coli*. Antimicrobial activity for the methanol extract of *E. hirsutum* against the *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Mycobacterium smegmatis* was found to be 12 mm, 12 mm, and 16 mm inhibition zone, respectively. Methanol extract gave moderate activity against the *Yersinia pseudotuberculosis*, *Staphylococcus aureus*, and *Bacillus cereus* within 6-8 mm, respectively. Antioxidant activity of water extract was found to be 12.77 ± 0.02 μM (CUPRAC) and 0.034 mg/mL (IC_{50}, DPPH). IC_{50} values for the tyrosinase enzyme inhibitory activity for the methanol and water extracts were determined to be 0.20 ± 0.01 μg/mL and 0.16 ± 0.09 μg/mL, respectively.

Keywords: *Epilobium hirsutum*, essential oil, GC-FID/MS, SPME, antimicrobial, antioxidant, tyrosinase activity

1. Introduction

The *Epilobium* genus is represented by more than 185 herbaceous perennial species belongs to the Onagraceae family [1,2] which are distributed in North Africa, most of Europe, parts of Asia, North America, and Australia. It grows in wet habitats up to 2,500 meters above sea level [1,2]. The traditional name of *Epilobium* species is "Yaki Otu" in Turkey. Young shoots of *Epilobium* species are consumed as food. These species have been used in traditional medicine for the treatment of mouth wounds [3]. An ointment that is prepared from the leaf of *Epilobium* species has been used for skin disorders. *Epilobium* taxa are one of the best known traditional used medicinal plants for prostate and gastrointestinal disorders in Turkey and worldwide [4-9]. *Epilobium hirsutum* L. (syn. Chamaenerion *hirsutum* (L.) Scop.) is native throughout the world [1,2]. The chemical constituents of *Epilobium* species have been described and polyphenolic compounds (phenolic acids, steroids, tannins, and flavonoids) were the main constituents occurring in *Epilobium* herb [10-22]. The extracts of *Epilobium* taxa have been reported to exhibit antiproliferative [21], antiaging [22], antioxidants [23-26], anti-inflammatory [27], antimicrobial [28-32], analgesic, prostate cancer healer [33,34], anti-proliferative [21], antinociceptive [35], anti-diarrhoeal, anti-motility, and anti-secretory [36] properties. The pharmacological and therapeutic potentials of *E. hirsutum* have given as a review report [4, 6]. In the

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literature, the chemical composition and antibacterial activity (*Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enterica*, and *Escherichia coli*) of the essential oil of *E. hirsutum* from Iran were mentioned and the most abundant component was reported as pulegone, constituting 74.6% of the oil [37]. The volatile contents for SPMEs extracts, tyrosinase, and some of the antimicrobial effects (nine microorganisms) of the essential oil and solvent extracts of *E. hirsutum* growing in Turkey have not been previously reported. This work aims to analyze the chemical variation, antimicrobial, antioxidant, and tyrosinase activities of the EO and solvent extracts (methanol and water) of *E. hirsutum*. According to our literature survey, this is the first comparative study of the phytochemical composition of *E. hirsutum* growth in Turkey. Such a study is needed to show how the different extraction methods affect the volatile constituents and different chemotypes.

2. Experimental

2.1. Plant material

Wild grown *E. hirsutum* was collected from 1450 m above sea level in August 2018 from Koyulhisar-Sivas (SE part of Turkey). The fresh plant materials air-dried in the shade at room temperature and analyzed as soon as possible. The plant was authenticated by Prof. K. Coşkunçelebi by using Flora of Turkey [2]. The voucher specimen was deposited and stored in the Herbarium of Biology, Karadeniz Technical University.

2.2. Hydrodistillation apparatus and procedure

Dried grounded aerial part of *E. hirsutum* (125 g) was used to obtain essential oil by hydrodistillation (HD) using a modified Clevenger-type apparatus with a cooling bath (-15°C) system (3h) (yield (w/w): 18.3 mg). The HD oil was extracted with *n*-hexane (HPLC grade, 0.5 ml) and dried over anhydrous Na_2SO_4, and kept in sterilized dark glass bottles in the refrigerator at 4 °C before the analysis [38-39].

2.3. *n*-Hexane, methanol, and water extracts of *E. hirsutum*

Dried grounded plant (5 g, each) was put into three different flasks (50 mL) and extracted three times with an analytical grade *n*-hexane, methanol, and water solvents (10 ml × 3; 12 h each), respectively. After the suction filtration, the same extracts were combined. *n*-Hexane and methanol were evaporated at the 40 °C to give crude *n*-hexane (46.2 mg) and methanol (96.5 mg). Water was lyophilized to obtain crude water extract (75 mg) [38].

2.4. Solid-phase microextraction (SPME) analysis

The blended dried plant (1.2 g each) and *n*-hexane extract (30 mg) of *E. hirsutum* were placed in a sealed SPME vial (10 mL) with a silicone-rubber septum cap then submitted to a SPME device (Supelco, USA). A DVB/Carboxen/PDMS coating fiber was employed to receive volatile components. The condition of SPME fibers was carried out for 5 min at 250 °C in the GC injector. Extraction was made with magnetic stirring at 80 °C. The incubation and extraction time were 5 and 10 min, respectively. Fiber with extract of volatile compounds was subsequently injected into the GC injector. GC-FID/MS analyzes were performed using a Shimadzu QP2010 Ultra mass selective detector attached to the 2010 Plus chromatograph. Helium was employed as a carrier gas at a flow rate of 1 mL/min. The injection was carried out in split mode (1:30) at 230 °C. The sample was analyzed and reported. The temperature, incubation, and extraction time were optimized according to the studies in the literature [38-40].

2.5. Gas chromatography-Mass spectrometry (GC-FID/MS)

EO analysis was carried out using a Shimadzu QP2010 ultra GC-FID/MS, Shimadzu 2010 plus FID, fitted with a PAL AOC-5000 plus autosampler, and Shimadzu Class-5000 Chromatography Workstation software. The separation was analyzed using a Restek Rxi-5MS capillary column (30 mm × 0.25 mm × 0.25 μm) (USA). Essential oil injection to GC-FID/MS was performed in split mode (1:30) at 230 °C. The essential oil solution (1 μL) in *n*-hexane was injected and analyzed with the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp and the final temperature of 250 °C was held for 4 minutes. Helium (99.999 %) was used as carrier gas with a constant flow-rate of 1 mL/min. Detection was determined in electronic impact mode (EI); ionization voltage was at 70 eV, and scan mode (40-450 *m*/*z*) was used for mass acquisition. Samples were analyzed and mean reported [41-42].

2.6. Identification of volatile constituents

Retention indices and chromatographic peaks were used to identify the volatile constituents. Retention indices were compared to C_6-C_{32} alkane standards. Individual chromatographic peaks in the mass spectra were compared with the commercial libraries (FFNSC1.2, W9N11, and NIST) [38-43].

2.7. Antimicrobial activity assessment (Agar-well diffusion method)

All test microorganisms which were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) were *Bacillus cereus* 709 ROMA, *Candida albicans* ATCC

60193, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Mycobacterium smegmatis* ATCC607, *Pseudomonas auroginosa* ATCC 27853, *Saccharomyces cerevisiae* RSKK 251, *Staphylococcus aureus* ATCC 25923, and *Yersinia pseudotuberculosis* ATCC 911. T,he plant extracts were dissolved in *n*-hexane, methanol, and water to prepare extracts stock solution. Antimicrobial susceptibility of the EO, *n*-hexanes, methanol, and water extracts of *E. hirsutum* were screened using the agar-well diffusion method [44-45]. Each bacterium and the yeast were cultured in Mueller Hinton (MH) (Difco, Detroit, MI) broth and yeast extract broth, respectively. Then the microorganisms were diluted nearly 106 colony-forming unit (cfu) per mL. For yeast-like fungi, Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) was used. Microorganisms were "flood-inoculated" onto MH and SD agars and dried under aseptic conditions. 50 µL of essential oil, solvent extracts of *E. hirsutum*were delivered into wells (diameter= 5 mm) opened on agar plates, and incubated at 35 °C for 18h. The growing of *Mycobacterium smegmatis* was made for 3 to 5 days on MHA plates at 35 °C. Microbial activity was evaluated by measuring the 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. All tests were carried out in triplicates.

2.8. Determination of antioxidant capacity (CUPRAC and DPPH)

In vitro antioxidant property of *E. hirsutum*aqueous extract was tested using 2,2-diphenyl-1-picrylhydrazyl scavenging (DPPH). Cupric reducing activity known as cupric reducing antioxidant capacity (CUPRAC) methods. CUPRAC assay was performed according to the literature [46]. In a test tube, 1 ml of each 1.0×10^{-2} M $CuCl_2$, 7.5×10^{-3} M neocuproine and 1.0 M ammonium acetate buffer (pH 7.0), and 1.1 mL of *E. hirsutum* aqueous extract at different concentrations was mixed together to make the final volume 4.1 mL. After 30 minutes incubation period, the absorbance was read 450 nm using Shimadzu UV-1600 spectrophotometer. Trolox was employed as a reference standard in the measurements. Results were expressed as µmol Trolox/g dry weight of *E. hirsutum* extract (µmol Trolox/g DW). The DPPH radical scavenging capacity for the aqueous extract of *E. hirsutum* was tested by the spectrometric method. The assay mixture (1.5 mL), methanolic DPPH solution (0.75 mL, 0.1 mM) and different concentrations of extracts (0.75 mL) was prepared. The mixture was incubated in the dark at room temperature for 50 min and absorbance was measured at 517 nm using Shimadzu UV-1600 spectrophotometer [47]. The assay was also performed for a control mixture without *E. hirsutum* extract but with extraction solvent. Trolox was used as a positive control. The following equation was used to calculate the inhibition of DPPH radical activity.

$$\%\ Inhibition = \% = \left[1 - \left[\frac{(A_{sample} - A_{sample\ blank})}{A_{control}}\right]\right] x100$$

2.9. Tyrosinase inhibition assay

The tyrosinase inhibition for the aqueous, methanol, *n*-hexane extracts and essential oil of *E. hirsutum* was determined by pursuing the method described in the literature [48] with some modifications. 800 µL phosphate buffer (50 mM, pH 6.8), 15 µL mushroom tyrosinase (2500 U/mL) (T3824 SIGMA Tyrosinase from mushroom), and plant extract at different concentrations were mixed and incubated at room temperature for ten minutes. At the end of the incubation period 100 µL of 10 mM L-DOPA (3,4-dihyroxy-L-phenylalanine) solution was added and incubated for 30 minutes at room temperature. The absorbance of the formed dopachrome was measured at 470 nm with references at 700 nm using Shimadzu UV-1600 spectrophotometer. Kojic acid was used as a positive control. The extract concentration giving 50% (IC50) of the original tyrosinase activity was determined.

3. Results and discussion

3.1. Chemical composition of the EO and SPME

GC-FID/MS analysis of the EO, SPME, and SPME of *n*-hexane extract of *E. hirsutum* revealed a total of 35, 20, and 32 volatile compounds, representing 98.2%, 97.6%, and 98.8%, respectively. The volatile organic compounds of the EO, and SPMEs of *E. hirsutum*, their retention indices and percentages are listed in Table 1. Volatile compounds have been listed in the order of elution on the Rxi-5MS column used (24-27), which were identified by comparison of the registered mass spectrum libraries (NIST, Wiley7NL, FFNSC1.2, and W9N11), and by using the Kovats index [28-34]. A total of 77 volatile compounds were detected in the EO and SPMEs of *E. hirsutum* by the GC-FID/MS analysis. As was expected, the higher diversity of compounds was determined in the EO rather than SPMEs (Table 1).

The qualitative and quantitative differences were observed between EO and SPMEs due to the different extractions used. The identified constituents involve different classes of chemical compounds, including monoterpenes, monoterpenoids, sesquiterpenes, aromatic hydrocarbons, aliphatic hydrocarbons, terpene related compounds, aldehydes, ketones, esters, alcohols, acids, and other hydrocarbons.

Table 1. Identified VOCs form the EO and SPMEs of *E. hirsutum* growing in Turkey

No	Compounds	RI*	RI[a]	(%)[b] A1	A2	A3
1	Toluene	782	782	4.1	**-**	6.8
2	Octane	800	802	**-**	**-**	0.1
3	Capronaldeyhde	802	804	4.4	0.8	-
4	Butyl acetate	814	813	-	-	0.5
5	(*E*)-2-Hexenal	852	853	1.8	1.4	-
6	(*Z*)-3-Hexenol	865	860	46.5	10.3	-
7	Ethylbenzene	871	870	-	-	1.2
8	Hexanol	863	863	-	11.1	-
9	4-Methy-1-penten-3-ol	870	874	5.0	-	-
10	1,4-Dimethylbenzene	878	878	-	-	7.0
11	2,3-Dimethyl-3-butenol	894	895	-	-	1.3
12	Cyclohexanone	903	903	-	-	38.0
13	Heptanal	906	906	0.6	1.6	-
14	1-Methylethylbenzene	929	929	-	-	0.1
15	*α*-Pinene	940	939	1.7	-	-
16	3-Ethyl-2-methylheptane	942	941	-	-	0.1
17	Propylbenzene	960	957	-	-	2.0
18	(*E*)-2-Heptanal	959	958	1.0	-	-
19	1-Ethyl-3-methylbenzene	968	965	-	-	10.8
20	Heptanol	959	959	**-**	6.7	-
21	1-Ethyl-4-methylbenzene	970	971	-	-	3.9
22	*β*-Pinene	978	972	0.5	-	-
23	1-Etil-2-metilbenzen	979	983	-	-	2.8
24	6-Methyl-5-heptene-2-one	981	977	0.9	-	-
25	2-Pentylfuran	993	992	0.4	6.7	-
26	1,3,5-Trimethylbenzene	996	997	-	-	17.0
27	Octanal	998	1002	-	0.5	-
28	(*Z*)-3-Hexenyl acetate	1004	1005	**-**	18.2	-
29	Hexyl acetate	1010	1011	**-**	3.3	-
30	(*E,E*)-2,4-Heptadienal	1012	1012	0.2	-	0.2
31	*p*-Cymene	1012	1016	0.5	-	-
32	1,2,4-Trimethylbenzene	1035	1036	-	-	3.0
33	Limonene	1031	1034	0.1	1.4	-
34	Benzene acetaldeyhde	1036	1036	5.1	16.8	-
35	Indane	1041	1040	**-**	-	0.5
36	Eucalyptol	1046	1046	0.3		-
37	(*Z*)-*β*-Ocimene	1041	1046	-	1.7	-
38	1-Methyl-3-propylbenzene	1053	1052	-	–	0.6
39	1,4-Dimethylbenzene	1056	1056	-	-	0.3
40	1-Ethyl-3,5-dimethylbenzene	1058	1059	-	-	0.5
41	3-Methyldecane	1072	1066	-	-	0.1
42	1-Methyl-4-propylbenzene	1061	1068	-	-	0.1
43	Octanol	1063	1059	-	0.6	-
44	4-Ethyl-1,2-dimethylbenzene	1077	1078	-	-	0.3
45	1-Ethyll-2,4-dimethylbenzene	1083	1080	-	-	0.2
46	2-Ethyll-1.4-dimethylbenzene	1085	1086	-	-	0.3
47	Undecane	1100	1095	-	-	0.6
48	Nonanal	1101	1102	1.7	5.8	-
49	1-Ethyl-2,3-dimethylbenzene	1113	1108	-	-	0.1
50	1,2,4,5-Tetramethylbenzene	1131	1132	-	-	0.1
51	Camphor	1161	1161	1.1	-	-
52	Nonanol	1171	1168	-	2.1	-
53	*α*-Phellandren-8-ol	1170	1170	0.5	-	-
54	Naphthalane	1181	1179	-	-	0.1
55	Terpinol-4-ol	1192	1191	1.7	-	-
56	*α*-Terpinol	1191	1193	2.3	-	-
57	Dodecane	1200	1204	-	-	0.1
58	Decanal	1201	1202	1.4	2.8	-
59	*β*-Cyclocitral	1220	1225	0.4	-	-
60	2-(*E*)-Decenal	1260	1260	0.3	-	-
61	(*Z*)-3- Hexenyl tiglate	1319	1321	-	1.0	-
62	(*E*)-*β*-Damascenone	1383	1387	0.5	-	-
63	Tetradecane	1400	1403	-	-	0.1
64	(*E*)-Karofilen	1417	1417	-	4.2	-
65	Geranyl acetone	1453	1450	0.3	-	-
65	Geranyl acetone	1453	1450	0.3	-	-
66	(*E*)-Ethyl cinnamate	1465	1467	-	0.6	-
67	(*E*)-*β*-ionone	1487	1489	1.0	-	-
68	Pentadecane	1500	1503	0.8	-	-
69	*β*-Bisabolene	1509	1508	-	-	0.1
70	Hexadecane	1600	1602	-	-	0.1
71	Hexahydrofarnesyl acetone	1847	1850	0.7	-	-
72	Nonadecane	1900	1901	0.5	-	-
73	Heptadecanone	1901	1906	0.3	-	-
74	Hexadecanoik acid	1966	1963	0.2	-	-
75	Manoyl oxide	1989	1993	0.2	-	-
76	Heneicosane	2100	2100	1.1	-	-
77	Tricosane	2300	2299	10.1	-	-

Chemical classes;	% [b] and NC[c]		
Monoterpene hydrocarbons	2.3:3	3.1:2	-
Monoterpenoids	6.3:6	-	-
Sesquiterpenes hydrocarbon	-	4.2:1	0.1:1
Aromatic hydrocarbons	5.0:3	6.7:1	57.1:19
Aliphatic hydrocarbons	12.0:3	-	1.3:8
Terpene related to compounds	2.7:5	1.0:1	-
Aldehydes	16.5:9	29.7:7	-
Ketone	1.7:3	-	38.0:1
Esters	-	18.8:2	0.5:1
Alcohols	51.5:2	30.8:5	1.3:1
Acids	0.2:1	-	-
Others	-	3.3:1	0.5:1
Total	98.2:35	97.6:20	98.8:32

* Retention Index of references; [a] Retention Index calculated with the RT relative to that of n-alkane (C_6-C_{32}) series; [b] Percentages were obtained by FID peak-area normalization; [c] NC: Number of compounds; A1: HD; A2; SPME; A3: SPME *n*-hexane extract

The extractions of *E. hirsutum* had two effects on the composition of the plant, which are the quantitative changes of compounds and appearance/disappearance of volatiles. Some of the compounds, like 4-methy-1-penten-3-ol, (*E*)-2-heptanal, *β*-pinene, *p*-cymene, *α*-phellandren-8-ol, terpinol-4-ol, *β*-cyclocitral, 2-(*E*-decenal, *(E)-β*-damascenone, geranyl acetone, *(E)-β*-ionone, pentadecane, hexahydrofarnesyl acetone, nonadecane, heptadecanone, hexadecanoic acid, manoyl oxide, heneicosane, and tricosane were found only in EO of *E. hirsutum*. Whereas, hexanol, heptanol, octanal, (*Z*)-3-hexenyl acetate, hexyl acetate, (*Z*)-*β*-ocimene, octanol, nonanol, (*Z*)-3-hexenyl tiglate, (*E*)-carofilen, and (*E*)-ethyl cinnamate were existed only in the SPME of *E. hirsutum.* Octane, butyl acetate, ethylbenzene, 1,4-dimethylbenzene, 2,3-dimethyl-3-butenol, cyclohexanone, 1-methylethylbenzene, 3-ethyl-2-methylheptane, propylbenzene, 1-ethyl-3-methylbenzene, 1-etil-2-metilbenzen, 1,2,4-trimethylbenzene, indane, 1-methyl-3-propylbenzene, 1,4-dimethylbenzene, 1-ethyl-3,5-dimethylbenzene, 3-methyldecane, 1-methyl-4-propylbenzene, 4-ethyl-1,2-dimethyl-benzene, 1-ethyll-2,4-dimethylbenzene, 2-ethyll-1.4-dimethylbenzene, undecane, 1-ethyl-2,3-dimethylbenzene, 1,2,4,5-tetramethyl-benzene,

naphthalane, dodecane, tetradecane, β-bisabolene, and hexadecane were present only in the SPME of *n*-hexane extract of *E. hirsutum.* The quantitative composition of volatiles varied depending on the sample origin. Possibly the different extraction methods (HD and SPME) cause the variation of chemical components as in the literature [42-46]. Thus, the existence of different chemotypes in the EO, SPME, and SPME of *n*-hexane extract of *E. hirsutum* were identified. Comparative analysis for the extracts of the plant showed that the amount of alcohol (HD, 51.5% vs SPME 30.8%) was the major group of compounds in EO and SPME. Despite these, aromatic hydrocarbons were found to be major constituents of SPME of *n*-hexane extract of *E. hirsutum.* The numbers of the identified terpenes/terpenoids were greater in EO of *E. hirsutum.*

In the literature, the EO of *E. hirsutum* gave pulegone (74.6%), menthofuran (11.8%), and 1,8-cineole (2.4%) as the major constituents which we could not found. The other compounds were also so different than in our analysis [37]. The antibacterial activity of the EO has been mentioned against four bacterial species (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli,* and *Salmonella enterica*) and inhibition zone was 10 mm (3.1%), 10 mm (3.1%), 10 mm (25%) and 10 mm (50%), respectively [37]. In the literature, *in vivo* and *in vitro* ethnopharmacological uses of *Epilobium angustifolium*, *E. stevenii*, and *E. hirsutum* explained and the probable wound-healing activity mechanism was mentioned [5]. A study related to the determination of sesquiterpene derivatives from the essential oil of *E. hirsutum* was also reported [12]. In the *E. hirsutum* seed oil palmitic, stearic, oleic, linoleic, γ-linolenic, and α-linolenic acids were reported and palmitic and linoleic acids were mentioned to be dominant compounds [15].

It is known that the reduced cost of extraction is advantageous for the SPME method in terms of decomposition, time, and energy. The variations in the VOCs on *Epilobium* taxa may be due to environmental, storage, and analysis conditions. Results of this work, extractions methods that used had remarkable changes in volatiles. The differences in volatile composition were also demonstrated by the literature [49-50]. However, using the different extraction methods let the appearance of new compounds had a positive effect on the quality of *E. hirsutum*. (*Z*)-3-Hexene-1-ol (46.5%), (*Z*)-3-hexenyl acetate (18.2%), and cyclohexanone (38%) were major compounds for the EO, SPME, and SPME of *n*-hexane of the *E. hirsutum*, respectively. It was found that only the EO of *E. hirsutum* rich in monoterpenes/monoterpenoids which showed significant inhibitory activity against some fungi, like *E. coli*, *E. faecalis, C. albicans, and S. cerevisiae.* However, no activity was reported against the *Y. pseudotuberculosis, P. aeruginosa, S. aureus, B. cereus,* and *M. smegmatis.* The evaluation of antimicrobial activities for the pure compounds of *E. hirsutum* was beyond the scope of this work. However, according to the experimental results and the above mentioned published data [4-37], the volatile compounds and solvent extracts of *E. hirsutum* also could be of therapeutic value.

3.2. Biological activities

3.2.1. Antimicrobial activity

Antimicrobial activities of the EO and solvent extracts (*n*-hexane, methanol, and water) of *E. hirsutum* were screened by using the agar well diffusion method with the microorganisms against *Escherichia coli*, *Yersinia pseudotuberculosis*, *P. auroginosa, Enterococcus faecalis*, *S. aureus, B. cereus*, *M. smegmatis, C. albicans*, and *Saccharomyces cerevisiae* [44-45]. In general, EO and methanol extract showed moderate antimicrobial activities with the inhibition zone in the range of 6-16 mm against *E. coli, Y. pseudotuberculosis, P. aeruginosa, E. faecalis, S. aureus, B. cereus, M. smegmatis, C. albicans*, and *S. cerevisiae*, respectively (Table 2).

Table 2. Antimicrobial activity of the EO and methanol extract of *E. hirsutum.*

Sample Extracts	Const. (µg/ml)	Microorganisms and inhibition zone (mm)								
		Gram (-) Bacteria			Gram (+) Bacteria			No G	Yeast Mush.	
		Ec	*Yp*	*Pa*	*Ef*	*Sa*	*Bc*	*Ms*	*Ca*	*Sc*
EO	18.26	10	-	-	6	-	-	-	6	6
CH_3OH	96.50	-	8	12	12	6	8	16	-	-
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 against *E. coli* with 10 mm inhibition. The methanol extract of gave better activity against the *P. aeruginosa*, *E. faecalis*, and *M. smegmatis* with 12, 12, and 16 mm inhibition zones, respectively. Therefore, the bactericidal activity of EOs and the solvent extract obtained from *E. hirsutum* may be mainly related to the high content of alcohols and aldehydes. Other compounds (Table 1), which were also present in the samples, were reported to have antibacterial activities, may also collectively have a remarkable contribution to the bactericidal activities of the EO and methanol extracts. The antibacterial activity variations may be due to factors such as composition and concentration of EO and solvent extracts. The *n*-Hexane and water extracts of *E. hirsutum* did not show antimicrobial activity against all tested microorganisms. In the literature, antibacterial, antifungal, antioxidant activities, total phenolic content, postmenopausal effect,

and prostate cancer activity for the solvent extracts of *E. hirsutum* had reported [34].

3.2.2. *Antioxidant (CUPRAC and DPPH) activities*

CUPRAC is a method used to measure total antioxidant capacity based on the Copper (II) reduction capacity of antioxidants [50]. In the CUPRAC method, a concentration-absorbance graph for Trolox as the reference standard and aqueous extract of *E. hirsutum* were generated. The slopes obtained from the graphs of the sample were scaled to the slope of the standard graph of Trolox. The Trolox Equivalent Antioxidant Capacity (TEACCUPRAC) value was calculated to be 12.77 ± 0.02 (μmol Trolox/g DW). The DPPH is a stable free radical antioxidant method that produces a violet color in methanol, shows maximum absorption at wavelength 515-520 nm. When DPPH methanol solution meets an antioxidant, its color is reduced to yellow [51]. THE remaining DPPH radical in a reaction mixture is gives the radical scavenging potential of a sample. When the DPPH method was applied to *E. hirsutum* aqueous extract the IC_{50} was calculated to be 0.034 ± 0.002 mg/mL. The IC_{50} calculated for the positive standard Trolox is 0. 120 ± 0.008 mg/mL. CUPRAC and DPPH antioxidant capacity revealed that *E. hirsutum* aqueous extract seems to have a high radical scavenging effect due to the poly phenolic compounds [12-20] when compared to the Trolox standard.

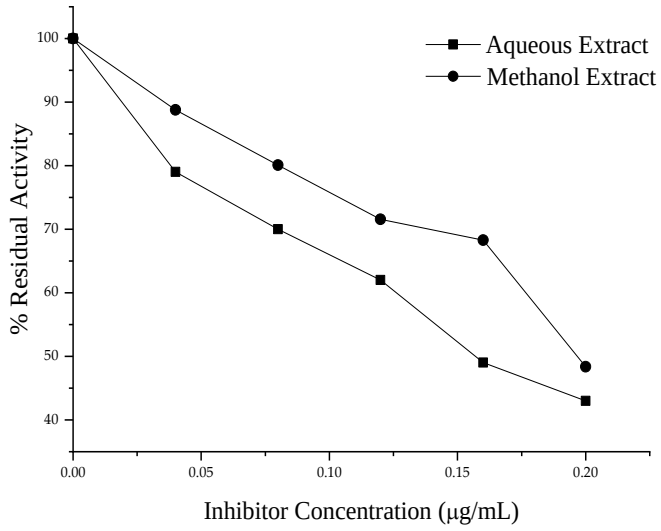

Figure 1. Tyrosinase activity for the methanol and water extracts of *E. hirsutum* growing in Turkey

3.2.3. *Tyrosinase activity*

Tyrosinase inhibitors have commercial potential in cosmetic industries because of their capability to reduce the melanization of human skin [48]. The aqueous, methanol, *n*-hexane extracts, and essential oils of *E. hirsutum* were monitored for their mushroom tyrosinase inhibition potentials. *n*-Hexane extract and essential oil did not possess any effect against tyrosinase. The aqueous and methanol extracts of *E. hirsutum* inhibited the mushroom tyrosinase at different ratios (Figure 1). The results showed that aqueous and methanol extracts of *E. hirsutum* had dose-dependent inhibitory effects on mushroom tyrosinase activity. The IC_{50} values for tyrosinase inhibition were obtained as 0.20 ± 0.01 μg/mL and 0.16 ± 0.09 μg/mL for methanol and aqueous extracts, respectively. The IC_{50} value for the reference compound (Kojic acid) was 3.10 ± 0.20 μg/mL. When we compare our results with the tyrosinase inhibitory activity for the leaf extracts of *Hyaenanche globosa, Myrsine africana,* and *Aristotelia chilensis*, it is seen that there is a better tyrosinase effect [51, 52].

4. Conclusion

A total of 77 constituents were characterized by the EO and SPMEs of the plant which showed variation to contain different volatile compounds. The amounts of terpenoid compounds identified by EO and SPME were greater in SPME of *n*-hexane extract of *E. hirsutum*. (Z)-3-Hexene-1-ol (46.5%), (Z)-3-hexenyl acetate (18.2%), and cyclohexanone (38%) were the major compounds for the EO, SPME, and SPME of *n*-hexane of the *E. hirsutum* which showed that different extraction methods used, gave different components as in the literature. The EO and methanol extract gave activity against the *E. coli, Y. pseudotuberculosis, P. aeruginosa, E. faecalis, S. aureus, B. cereus, M. smegmatis, C. albicans, and S. cerevisiae* within the range of 6-16 mm, respectively. In general, the greatest activity of the EO was observed 10 mm value against *E. coli*. coli. Methanol extract gave better antimicrobial activity against the M. smegmatis was determined to be 16 mm value. Therefore, antioxidant antimicrobial and tyrosinase activities' overall results suggest that EO and solvent extracts of *E. hirsutum* may have promising prospects for pharmaceutical, food, and other industrial applications. In a further study, activity guided isolation and purification could be carried out.

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Disclosure statement

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

References

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

[2] D. Chamberlain, P.H. Raven, *Epilobium* L., Flora of Turkey and The East Aegean Island, Editor: P.H. Davis, 1972, Scotland, Edinburgh University Press.
[3] N. Zeybek, U. Zeybek, Pharmaceutical Botany, 1994, Turkey, Ege University Faculty of Pharmacy Publication.
[4] T. Ege, The pharmacological and therapeutic potentials of *Epilobium hirsutum* L., Int J Pharm Sci Rev Res, 57, 2019, 20-23.
[5] S. Karakaya, I. Suntar, Ö.F. Yakinci, O. Sytar, S. Ceribasi, B. Dursunoglu, H. Ozbek, Z. Guvenalp, In vivo bioactivity assessment on *Epilobium* species: A particular focus on *Epilobium angustifolium* and its components on enzymes connected with the healing process, J Ethnopharmacol, 262, 2020, 113-207.
[6] S. Granica, J.P. Piwowarski, M.E. Czerwińska, A.K. Kiss, Phytochemistry, pharmacology and traditional uses of different *Epilobium* species J. (Onagraceae): a review, J Ethnopharmacol, 156, 2014, 316-346.
[7] L. Pirvu, V. Nicorescu, C. Hlevca, D.I. Udeanu, I. Nicorescu, Antimicrobial and synergistic activity of some whole and selective *Epilobium hirsutum* L. (great willowherb) extracts tested on standard and wild *Staphylococcus aureus* strains, Farmacia, 63, 2015, 690-695.
[8] A. Vitalone, J. McColl, D. Thome, L.G. Costa, B. Tita, Characterization of the effect of *Epilobium* extracts on human cell proliferation, Pharmacology, 69, 2003, 79-87.
[9] A. Sayik, A.S. Yusufoğlu, A. Leyla, G. Türker, B. Aydin, L. Arslan, Dna-binding, biological activities and chemical composition of wild growing *Epilobium angustifolium* L. extracts from Çanakkale, Turkey, J Turkish Chem Soc, 4, 2017, 811-840.
[10] I.A. Schepetkin, A.G. Ramstead, L.N. Kirpotina, J.M. Voyich, M.A. Jutila, M.T. Quinn, Therapeutic potential of polyphenols from *Epilobium angustifolium* (Fireweed), Phytother Res, 30, 2014, 1287-1297.
[11] S. Jürgenson, V. Matto, A. Raal, Vegetational variation of phenolic compounds in *Epilobium angustifolium*, Nat Prod Res 26, 2012, 1951- 1953.
[12] I. Nitescu, D. Cucu, D. Lapa, O. Contz, Studies on a new method for photochemical screening using chromatography of medicinal plants, Farmacia, 42, 1994, 41-43.
[13] D. Cando, D. Morcuende, M. Utrera, M. Estevez, Phenolic-rich extracts from Willowherb (*Epilobium hirsutum* L.) inhibit lipid oxidation but accelerate protein carbonylation and discoloration of beef patties, Eur Food Res Technol 238, 2014, 741-751.
[14] I. Remmel, L. Vares, L. Toom, V. Matto, A. Raal, Phenolic compounds in five *Epilobium* species collected from Estonia, Nat Prod Commun, 7, 2012, 1323-1324.
[15] M. Pelc, O. Kosakowska, Z. Weglarz, J. Przybyl, A. Geszprych, Sterols and fatty acids in the seeds of evening primrose (Oenothera sp.) and willow herb (*Epilobium* sp.), Herba Pol, 51, 2005, 20-24.
[16] M.A. Nawwar, M.S. Marzouk, W. Nigge, M. Linscheid, High-performance liquid chromatographic/electrospray ionization mass spectrometric screening for polyphenolic compounds of *Epilobium hirsutum* - the structure of the unique ellagitannin epilobamide-A, J Mass Spectrom, 32, 1997, 645-654.
[17] J. De Pascual Teresa, B. Corrales, M. Grande, A review on presence of oleanolic acid in natural products, An Quim, 75, 1979, 135-139.
[18] S. Ivancheva, N. Manolova, J. Serkedjieva, V. Dimov, N. Ivanovska, Polyphenols from Bulgarian medicinal plants with anti-infectious activity, Basic Life Sci 59, 1992, 717-728.
[19] H.H. Barakat, S.A.M. Hussein, M.S. Marzouk, I. Merfort, M. Linscheid, M.A.M. Nawwar, Polyphenolic metabolites of *Epilobium hirsutum*, Phytochemistry, 46, 1997, 935-941.
[20] B.H. Tóth, B. Blazics, Á. Kéry, Polyphenol composition and antioxidant capacity of *Epilobium* species, J Pharmaceut Biomed, 49, 2009, 26-31.
[21] A. Vitalone, F. Bordi, C. Baldazzi, G. Mazzanti, L. Saso, B. Tita, Anti-proliferative effect on a prostatic epithelial cell line (PZ-HPV-7) by *Epilobium angustifolium* L., Farmaco, 56, 2001, 483-489.
[22] E. Ruszová, J. Cheel, S. Pávek, M. Moravcová, M. Hermannová, I. Matějková, J. Spilková, V. Velebný, L. Kubala, *Epilobium angustifolium* extract demonstrates multiple effects on dermal fibroblasts in vitro and skin photo-protection in vivo, Gen Physiol Biophys, 32, 2013, 347-359.
[23] V. Kaškonienė, M. Stankevičius, T. Drevinskas, I. Akuneca, P. Kaškonas, K.B. Survilienė , A.Maruška, O.Ragažinskienė, O. Kornyšova, V. Briedis, R. Ugenskienė, Evaluation of phytochemical composition of fresh and dried raw material of introduced *Chamerion angustifolium* L. using chromatographic, spectrophotometric and chemometric techniques, Phytochemistry, 115, 2015, 184-193.
[24] H.C. Onar, A. Yusufoglu, G. Turker, R. Yanardag, Elastase, tyrosinase and lipoxygenase inhibition and antioxidant activity of an aqueous extract from *Epilobium angustifolium* L. leave, J Med Plants Res, 6, 2012, 716-726.
[25] B.T. Hevesi, P.J. Houghton, S. Habtemariam, Á. Kéry, Antioxidant and anti-inflammatory effect of *Epilobium parviflorum* Schreb, Phytother Res, 23, 2009, 719-724.
[26] A.K. Kiss, A. Bazylko, A. Filipek, S. Granica, E. Jaszewska, U. Kiarszys, A. Kośmider, J. Piwowarski, Oenothein B's contribution to the anti-inflammatory and antioxidant activity of *Epilobium* sp., Phytomedicine, 18, 2011, 557-560.
[27] H. Juan, W. Sametz, A. Hiermann, Anti-inflammatory effects of a substance extracted from *Epilobium angustifolium*, Agents Actions, 23, 1988, 106-107.
[28] W.J. Bartfay, E. Bartfay, J.G. Johnson, Gram-negative and Gram-positive antibacterial properties of the whole plant extract of Willowherb (*Epilobium angustifolium*), Biol Res Nurs, 14, 2012, 85-89.
[29] L. Battinelli, B. Tita, M.G. Evandri, G. Mazzanti, Antimicrobial activity of *Epilobium* spp. Extracts, Farmaco, 56, 2001, 345-348.
[30] I. Kosalec, N. Kopjar, D. Kremer, Antimicrobial activity of Willowherb (*Epilobium angustifolium* L.) leaves and flowers, Curr Drug Targets, 14, 2013, 986-991.
[31] Q. Zeng, J. Wu, P. Lin, Chemical composition and antimicrobial activity of the essential oil from *Epilobium angustifolium*, Chem Nat Compd, 52, 2016, 1113-1115.
[32] S. Silló, E. Varga, Á. Belák, A. Maráz, Phytochemical and antimicrobial investigation of *Epilobium angustifolium* L., Acta Pharm Hung, 84, 2014, 105-110.
[33] B. Tita, H. Abdel-Haq, A. Vitalone, G. Mazzanti, L. Saso, Analgesic properties of *Epilobium angustifolium*, evaluated by the hot plate test and the writhing test, Farmaco 56, 2001, 341-343.
[34] M. Stolarczyk, M. Naruszewicz, A.K. Kiss, Extracts from *Epilobium* sp. herbs induce apoptosis in human hormone-dependent prostate cancer cells by activating the mitochondrial pathway, J Pharm Pharmacol, 65, 2013, 1044-1054.
[35] F. Pourmorad, M.A. Ebrahimzadeh, M. Mahmoudi, S. Yasini, Antinociceptive activity of methanolic extract of *Epilobium hirsutum*, Pak J Biol Sci, 10, 2007, 2764-2767.
[36] F. Vitali, G. Fonte, A. Saija, B. Tita, Inhibition of intestinal motility and secretion by extracts of *Epilobium* spp. in mice, J Ethnopharmacol, 107, 2006, 342-348.
[37] E. Ehsan, M.R. Akhgar, A. Kariminik, Chemical constituents and antibacterial activity of the essential oil from *Epilobium hirsutum*, J Biodivers Environ Sci, 7, 2015, 338-344.
[38] I. Erik, G. Kiliç, E. Öztürk, Ş.A. Karaoğlu, N. Yaylı, Chemical composition, antimicrobial and lipase enzyme activity of essential oil and solvent extracts from *Serapias orientalis* subs. *orientalis*, Turk J Chem, in press, 2020.
[39] G. Tosun, N. Kahriman, C. Albay, Ş.A. Karaoğlu, N. Yaylı, Antimicrobial activity and volatile constituents of the flower, leaf, and stem of *Paeonia daurica* grown in Turkey, Turk J Chem, 35, 2011, 145-153.

[40] G. Renda, A. Özel, B. Barut, B. Korkmaz, N. Yaylı, 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, 2019, 1831-1842.
[41] B. Yaylı, G. Tosun, M. Karaköse, G. Renda, N. Yaylı, 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, 2014, 2541-2544.
[42] N. Yaylı, A. Yaşar, N. Yaylı, M. Albay, K. Coşkunçelebi, Essential oil analysis and antimicrobial activity of *Paeonia mascula* from Turkey, Nat Prod Commun, 3, 2008, 941-944.
[43] R.P. Adams, Identification of essential oil components by gas chromatography/mass spectrometry (4. edition), 2007, USA, Allured publishing Corporation.
[44] G.L. Woods, B.A. Brown-Elliott, P.S. Conville, E.P. Desmond, G.S. Hall, G. Lin, G.E. Pfyffer, J.C. Ridderhof, S.H. Siddiqi, R.J. Wallace Jr, N.G. Warren, F.G. Witebskyet, Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes (2. Edition), 2011, USA, Clinical and Laboratory Standards Institute.
[45] A.L. Barry, W.A. Craig, H. Nadler, L. Barth Reller, C.C. Sanders, J.M. Swenson, Methods for determining bactericidal activity of antimicrobial agents; approved guideline (4. edition), 1999, USA, Clinical and Laboratory Standards Institute.
[46] R. Apak, K. Güçlü, B. Demirata, M. Özyürek, E.S. Çelik, B. Bektaşoğlu, I.K. Berker, D. Özyurt, Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay, Molecules, 12, 2007,1496-1547.
[47] M.S. Blois, Antioxidant determinations by the use of a stable free radical, Nature, 181, 1958, 1199-1200.
[48] R. Fu, Y. Zhang, Y. Guo, F. Chen, Antioxidant and tyrosinase inhibition activities of the ethanol-insoluble fraction of water extract of *Sapium sebiferum* (L.) Roxb. Leaves, S Afr J Bot, 93, 2014, 98-104.
[49] N.Y. Iskender, N. Yaylı, N. Yildrim, T.B. Cansu, S. Terzioglu, The volatile constituents of the flower, leaf, and stem of *Verbascum wiedemannianum* grown in Turkey, J Oleo Sci, 58, 2009, 117-121.
[50] O. Üçüncü, N. Yaylı, A. Yaşar, S. Terzioğlu, N. Yaylı, Chemical composition of the essential oils from flower, leaf, and stem of *Senecio trapezuntinus* Boiss. grown in Turkey, Nat Prod Commun, 3, 2008, 925-928.
[51] C.L. Cespedes, C. Balbontin, J. G. Avila, M. Dominguez, J. Alarcon, C. Paz, V. Burgos, L. Ortiz, I. Peñaloza-Castro, D.S. Seigler, I. Kubo, Inhibition on cholinesterase and tyrosinase by alkaloids and phenolics from *Aristotelia chilensis* leaves, Food Chem Toxicol, 109, 2017, 984-995.
[52] S. Momtaz, N.L. Basson, Inhibitory activities of mushroom tyrosine and DOPA oxidation by plant extracts, S Afr J Bot, 74, 2008, 577-582.