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Chemical constituents and antibacterial activity of the essential oil from *Epilobium hirsutum*

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Abstract *Epilobium* species have been traditionally used as medicinal plants for centuries. The present work studies the chemical composition and antibacterial activity of the essential oil of *Epilobium hirsutum* L. from Iran. The essential oil of the aerial parts of the plant was extracted by hydrodistillation method and analyzed by GC and GC/MS. Thirty-two compounds were identified in the essential oil, representing 99.8% of the total oil. The most abundant component was pulegone, constituting 74.6% of the oil. Furthermore, antibacterial activity of the oil was investigated using the disc diffusion method, with determination of minimum inhibitory concentration (MIC), against four bacterial including *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enterica* and *Escherichia coli*. The essential oil exhibited *in vitro* antibacterial activity against all tested bacterial strains. Minimum inhibitory concentrations of the oil on the growth of *S. aureus, B. cereus, S. enterica*, and *E. coli* were 3.1, 3.1, 50 and 25%, respectively. Conclusively, *E. hirsutum* can be considered as an herbal antibacterial agent.

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Introduction

The genus *Epilobium* (Onagraceae) is represented by more than 200 species distributed worldwide (Granica *et al*., 2014). The most known species are *E. angostifolium* L., *E. parviflorum* Schreb. and *E. hirsutum* L., perennial herbs generally named Willowherb, with reference to the willow-like nature of their leaves (Battinelli *et al*., 2001). The Iranian Flora consists of 19 species of *Epilobium*, growing in different parts of the country (Mozaffarian, 2006). The name of *Epilobium* derives from the Greek words "epi" (upon) and "lobos" (a pod); the plant is so called because the flowers are arranged upon long, thin, pod-like seed cases. Some species of the genus are traditionally used internally for prostate and gastrointestinal disorders and externally as antiphlogistic and antiseptic remedies, to treat mycoses and to improve the healing of wounds (Battinelli *et al*., 2001).

The chemical composition of different *Epilobium* species and their bioactivities have been described. The studies showed that polyphenols were the main compounds occurring in *Epilobium* herb, among which flavonoids, phenolic acids and tannins were dominating constituents (Granica *et al*., 2014; Jürgenson *et al*., 2012; Tóth *et al*., 2009; Barakat *et al*., 1997). The extracts and isolated compounds from *Epilobium* species were shown to possess antimicrobial (Granica *et al*., 2014; Cosalec *et al*., 2013; Bartfay *et al*., 2012; Borchardt *et al*., 2008; Steenkamp *et al*., 2006; Battinelli *et al*., 2001), anti-proliferative (Vitalone *et al*. 2001), anti-inflammatory (Kiss *et al*., 2011; Hevesi *et al*., 2009), antinociceptive (Pourmorad *et al*., 2007), anti-diarrhoeal, anti-motility, anti-secretory (Vitali *et al*., 2006), analgesic (Tita *et al*., 2001) and antioxidant (Kiss *et al*., 2011; Tóth *et al*., 2009; Hevesi *et al*., 2009) activities.

Epilobium hirsutum, known as large-flowered Willowherb, is a perennial flowering plant widely distributed all over the world. It was used as an alternative remedy for the treatment of various diseases in ancient times. The medicinal parts of the plant are the herb and the roots that contain flavonoids, steroids and tannins (Granica *et al*., 2014; Pakravan *et al*., 2012). The present work studies both the chemical composition and antibacterial activity of the essential oil from the aerial parts of *E. hirsutum* growing wild in Iran for the first time.

Materials and methods

Plant material and essential oil isolation

The plant material was collected during the flowering stage from Gavehsoltani, the Gughar area, Kerman Province, Iran in June 2014. A voucher specimen (No. 8641) has been deposited in the Herbarium of Research Center of Agriculture and Natural Resources of Kerman, Iran. The air-dried aerial parts of the plant (200 g) were crushed and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus (Moosazadeh *et al*., 2014). The oil was dried over anhydrous sodium sulfate and stored in a tightly closed dark vial until the analysis. The yield of the oil was calculated based on dried weight of plant materials.

Essential oil analysis procedure

The constituents of the oil were analyzed by GC and GC/MS. GC analysis of the volatile components was carried out using a Hewlett-Packard 6890 instrument coupled to a flame ionization detector (FID). Compounds were separated on a HP-5 capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Helium was used as the carrier gas at a constant flow of 1 mL/min. The column temperature was kept at 60°C for 3 min and programmed to 220°C at a rate of 5°C/min. Injector and detector temperatures were kept at 250°C and 270°C, respectively. A mixture of aliphatic hydrocarbons (C_6–C_{23}) in hexane was directly injected into the GC injector under the above temperature programme in order to calculate the retention indices of each compound.

GC/MS analysis was performed using an Agilent 5975C mass spectrometer coupled to an Agilent 7890A gas chromatograph equipped with a HP-5MS capillary column (30 m × 0.25 mm, film thickness

0.25 µm). The carrier gas was helium, and the chromatographic conditions were as above. Spectrometer was scanned over the 40-400 amu range with an ionization voltage of 70 eV and an ionization current of 150 µA.

Identification of the components of the volatile oil was based on retention indices and computer matching with the Wiley and NIST libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Adams, 2004; Massada, 1976).

Bacterial cultures and antibacterial activity

The antibacterial activity of the oil was assessed against four bacterial species: *Staphylococcus aureus* (PTCC 1431), *Bacillus cereus* (PTCC 1015), *Salmonella enterica* (PTCC 1709) and *Escherichia* coli (PTCC 1339). The bacteria were cultured in Tripticase soy broth (Merck) for 24 h at 36°C ± 1°C. After 24 h of incubation, bacterial suspension (inoculums) was diluted with sterile normal saline (0.9%) to obtain 1.5×10^8 CFU/mL equal to 0.5 McFarland solution turbidity. The bacterial inoculums were spread using sterile cotton swab on Muller-Hinton agar media (Merck), separately. Antibacterial activity of the essential oil of *Epilobium hirsutum* on 4 bacterial strains was examined using disc diffusion method (Klancnik *et al.,* 2010). The oil was diluted by adding equal volume of Trypticase soy broth by serial double dilution technique. Blank discs (6 mm in diameter) with 20 µL of each dilution were impregnated on inoculated plates. Distilled water-loaded discs were used as negative controls. All plates were incubated for 24 h at 36°C ± 1°C. Antibacterial activity was assessed by measuring the diameter of the inhibition zone around the discs. The MIC values (Minimum concentration that inhibits the inoculum growth) of the essential oil against bacterial tested were determined as well.

Results

The aerial parts of *Epilobium hirsutum* yielded 0.8% (w/w) of a pale clear yellowish oil. The components of the oil are listed in Table 1, in which the percentage and retention indices (RI) of the components are given. As is shown, 32 compounds were identified in the essential oil of the plant, representing 99.8% of the total oil.

Table 1. Identified compounds in the essential oil of *Epilobium hirsutum*.

Compound	RI	Percent (%)
α-Pinene	934	0.6
Camphene	950	0.2
Sabinene	973	0.3
β-pinene	975	0.7
Myrcene	991	0.4
3-Octanol	996	0.2
α-Terpinene	1016	0.1
Limonene	1028	0.8
1,8-Cineole	1030	2.4
γ-Terpinene	1058	0.1
p-Mentha-3,8-diene	1070	0.1
Terpinolene	1087	0.1
3-Methyl butyl 2-methyl butanoate	1100	0.1
p-Menth-3-en-8-ol	1148	0.8
Menthone	1154	0.2
Menthofuran	1164	11.8
Borneol	1169	0.5
Isopulegone	1175	2.1
iso-Menthol	1184	0.2
neoiso-Menthol	1189	0.8
Pulegone	1238	74.6
Piperitone	1255	0.1
Menthyl acetate	1295	0.6
Piperitenone	1341	0.8
(*Z*)-Jasmone	1395	0.1
β-Caryophyllene	1418	0.3
Neryl acetone	1438	0.1
Germacrene D	1481	0.3
Spathulenol	1576	0.1
Caryophyllene oxide	1582	0.1
α-Cadinol	1654	0.1
Manoyl oxide	1994	0.1
Total identified	–	99.8

The most abundant constituent of the oil was pulegone (74.6%), followed by menthofuran (11.8%), 1,8-cineole (2.4%) and isopulegone (2.1%). Antibacterial potential of the essential oil was assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in Table 2. The essential oil showed *in vitro* antibacterial activity against all tested bacterial strains, especially Gram-positive ones. Minimum inhibitory concentration (MIC) and inhibition zone (IZ) of the essential oil on the growth of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella enterica* were (3.1%, 10 mm), (3.1%, 10 mm), (25%, 10 mm) and (50%, 10 mm), respectively.

Table 2. Dose response and antibacterial activity of the essential oil of *E. hirsutum*.

Oil concentration, IZ* / Bacteria	100%	50%	25%	12.5%	6.2%	3.1%	1.5%
Staphylococcus aureus	30	22	20	18	14	10	–
Bacillus cereus	30	20	15	12	10	10	–
Escherichia coli	15	12	10	–	–	–	–
Salmonella enterica	14	10	–	–	–	–	–

*Inhibition Zone (mm).

Discussion

Essential oils and extracts derived from plants have been used for many years ago in food preservation, pharmaceuticals, alternative medicine and natural therapies (Burt, 2004). It is necessary to investigate plants which have been used in traditional medicine to improve the quality of healthcare, because they have potential sources of novel antimicrobial compounds (Nalubega *et al.*, 2011). Several investigations have been conducted regarding *in vitro and in vivo* biological activities of plant essential oils and extracts.

In the present work, *In vitro* studies showed that the oil of *Epilobium hirsutum* inhibited bacterial growth, so that Gram-positive bacteria were more sensitive than Gram-negative ones. It has frequently been reported that Gram-positive bacteria are more susceptible to the essential oils than Gram-negative bacteria (Akhgar *et al.*, 2012; Ozturk and Ercisli, 2006; Mann *et al.*, 2000).

In another studies, ethanolic extracts of *E. hirsutum* has been investigated as anti-diarrheal remedies in several animal models (Vitali *et al.,* 2006). Flowers, stems and leaves extracts of *E. hirsutum* have been investigated for the antibacterial activity against several bacteria strains. It has been recognized that acetone and ethanolic extracts from all parts of the plant were able to inhibit the growth of *Bacillus cereus, B. subtilis, Staphylococcus aureus, S. epidermidis* and *Sarcina lutea* and only flower extracts of the plant have been effective on Gram-negative bacteria such as *Enterobacter aerogenes, Escherichia coli* and *Salmonella typhimurium* (Kunduhoglu *et al.*, 2011). In similar studies, the antiviral activity of *E. hirsutum* and *E. angustifolium* extracts against influenza viruses, especially H1N1 and H3N2 using both *in vitro* models and *in vivo* mouse model have been confirmed (Granica *et al.,* 2014).

As it can be seen from Table 1, the oil of *E. hirsutum* consisted of ten monoterpene hydrocarbons (3.4%), thirteen oxygenated monoterpenes (95.0%), two sesquiterpene hydrocarbons (0.6%), three oxygenated sesquiterpenes (0.3%), one oxygenated diterpene (0.1%) and three nonterpenoid compounds (0.4%). Consequently, oxygenated monoterpenes were the predominant fraction of the essential oil with pulegone as the main constituent. The antibacterial activity of the essential oil of *E. hirsutum* could be

associated to the presence of pulegone (Alim *et al.*, 2009; Bakkali *et al.*, 2008; Sonboli *et al.*, 2006; Ozturk and Ercisli, 2006; Duru *et al.*, 2004). Previous studies indicated that pulegone is bactericidal (Marinkovi *et al.*, 2002; Flamini *et al.*, 1999). Pulegone has a similar structure to carvone which has been shown to affect the cell membrane by dissipation of pH gradient and membrane potential of cells (Burt, 2004).

Conclusion

The present study suggests the oil as potential source of antibacterial compounds. Therefore, the essential oil from the aerial parts of *Epilobium hirsutum* could be a source of antibacterial agent required for therapeutic and food preservative applications.

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