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Composition and antimicrobial activity of the essential oil, distilled aromatic water and herbal infusion from *Epilobium parviflorum* Schreb.

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ABSTRACT

The essential oil of dry leaves and stems of *Epilobium parviflorum* Schreb. was obtained by hydrodistillation using a Clevenger apparatus, for 5 h with a 0.02% (w/w) yield. The qualitative and semi-quantitative analysis of its hexane extract by GC-FID and GC–MS showed the presence of 216 components and 123 constituents accounting for almost 94% of the oil were identified. GC–MS analysis revealed that the essential oil predominantly contains palmitic acid (30.8%), linoleic acid (12.5%) and α-linolenic acid (10.8%) as its major constituents. Analysis of aromatic water remained after hydrodistillation was performed; the main constituents were a group of monoterpenoids (38.9%). The antimicrobial activity of the distilled essential oil and the remaining aromatic water was evaluated against five microorganisms (*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*) using microdilution method. Results showed significant antibacterial activity against tested bacteria and yeast. The essential oil inhibited the growth of all tested bacteria and the minimum inhibitory concentration was determined to be around 10–40 μg mL^{-1}, even in the case of yeast it was 5 mg mL^{-1}. Distilled aromatic water contains much less substances but tests showed that it still has antimicrobial activity against all tested microbes (more on Gram-negative bacteria and yeast). Further, volatile profiles were also examined at other essential oils obtained by hydrodistillation of three pure herbal teas.

1. Introduction

Epilobium (*Oenotheraceae*), is an extensive genus with approximately 165 species worldwide. In Europe exist 28 species of the genus divided in two sections of *Epilobium* and *Chamaenerion* (Kiss et al., 2011). *Epilobium* species have been traditionally used as medicinal plants, e.g. remedies prepared from *E. angustifolium* L. possess anti-inflammatory properties, and extracts from *E. parviflorum* Schreb. relieve complaints associated with benign prostate hyperplasia of middle-aged and elderly men (Remmel et al., 2012). Published studies also refer about antioxidant (Kiss et al., 2011; Cando et al., 2014), anti-inflammatory (Juan et al., 1988; Hiermann et al., 1991), and antimicrobial (Kosalec et al., 2013; Bartfay et al., 2012; Battinelli et al., 2001; Eghmazi et al., 2015) activities of *Epilobium* species extracts.

Epilobium species contain biologically active compounds, such as tannins, flavonoids, polyphenolic acid and other phenolic compounds (Remmel et al., 2012; Jurgenson et al., 2012; Stolarczyk et al., 2013; Kiss et al., 2004). There is a study about chemical constituents of the essential oil (EO) from *Epilobium hirsutum* L. (Eghmazi et al., 2015), but there is no data about volatile compounds or essential oil composition of *Epilobium parviflorum* Schreb.

The aim of this study is to extend our knowledge about chemical constituents of *E. parviflorum* Schreb. and antimicrobial activity of essential oils from *E. parviflorum* Schreb., which is the most used *Epilobium* specie in remedies prepared for the treatment of prostate-associated ailments. We analysed herbal infusion prepared from dried *E. parviflorum* Schreb. because of its traditional using in folk medicine. Essential oil was studied as a potential substitution of herbal infusion. For this purpose the essential oils were extracted by hydrodistillation. Separation of essential oils and subsequent calculations of proportional representation of individual constituents have been carried out by gas chromatography with flame ionization detector (GC-FID). Identification of compounds

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Table 1
List of samples.

Sample	Origin/producer
Epilobium parviflorum Schreb., 2 kg – sample 1	*Mediate, s.r.o.* (Libchavy, Czech Republic)
Epilobium parviflorum Schreb., tea – sample 2	*Mediate, s.r.o.* (Libchavy, Czech Republic)
Epilobium parviflorum Schreb., tea packed in teabags – sample 3	*Valdemar Grešík* (Děčín, Czech Republic)
Epilobium parviflorum Schreb., tea packed in teabags – sample 4	*Herbex s.r.o.* (Hrašné, Slovakia)

has been carried out by gas chromatography/mass spectrometry system (GC–MS) and by comparing with linear retention indices. Antimicrobial activity of obtained essential oil and distilled aromatic water against several Gram-positive, Gram-negative bacteria and yeast was evaluated by a microdilution method as well. The importance of this study is in potential usage of essential oils from *E. parviflorum* Schreb. as an additive to food supplements.

2. Materials and methods

2.1. Plant material

In total, four samples of dried stems and leaves of *Epilobium parviflorum* Schreb. were analysed. A 2 kg sample of *E. parviflorum* Schreb. was purchased from Mediate, s.r.o. (Libchavy, Czech Republic). Other three samples were bought in local drugstores as pure herbal tea (see Table 1). All samples were commercially available products and *E. parviflorum* Schreb. was declared as single constituent. Dry weights of all samples were determined by analyser KERN MLB50-3 from Kern (Balingen, Germany) and ranged from 91% to 92%.

2.2. Chemicals and materials

n-Hexane, and *n*-alkane mixture standard solutions C8–C20 and C21–C40 in concentrations of 40 mg l^{-1} dissolved in *n*-hexane and in toluene, respectively, were purchased from Sigma-Aldrich (Prague, Czech Republic). Distilled water was purified using a Milli-Q® Water Purification System (Millipore SAS, Molsheim, France).

Solid phase microextraction (SPME) was performed with 100 μm PDMS fibre (Supelco, Bellefonte, PA, USA).

2.3. Bacterial cultures and cultivation

The following standard microorganisms or clinically isolated strain were used for the antimicrobial testing: Gram-positive bacteria (*Staphylococcus aureus* CCM 3953, *Enterococcus faecalis* PKN/079), Gram-negative bacteria (*Escherichia coli* CCM 3954, *Pseudomonas aeruginosa* CCM 3955), and yeast (*Candida albicans* CCM 8215). All bacterial cultures were cultivated onto Mueller-Hinton Agar (MHA, Himedia, Mumbai, India) under aerobic conditions for 24 h at 37 °C. Clinical yeast *Candida albicans* were cultivated onto Sabouraud Dextrose Agar (SDA, Himedia, Mumbai, India) under aerobic conditions for 72 h at 37 °C. Bacterial suspensions were prepared at a density of $\sim 10^8$ CFU mL^{-1} in physiological solution (8.5% (w/V) of NaCl in distilled water).

2.4. Sample preparation

2.4.1. Hydrodistillation of essential oils

All analysed plant samples were not treated before extraction. Treatment (drying and crushing) was done by producers. Hydrodistillation of essential oils was performed for 5 h, until no more essential oil was obtained.

Sample 1 was extracted by hydrodistillation in apparatus of Clevenger type (Kavalierglass a.s., Prague, Czech Republic). In total, 15 extraction runs were successively performed, each with 100 g of plant material and 1000 mL of water. Essential oils from each run were collected together. Distilled aromatic water, which remained after each extraction run in the return tube for recycling of aqueous part of the distillate (8 mL per one extraction run) was also collected. Chemical composition and antibacterial activity of essential oil of sample 1 and distilled aromatic water was conducted.

50 g of herbal tea (samples 2, 3, 4) was extracted with 500 mL water in the same apparatus as sample 1. Each extraction was performed three times.

2.4.2. Preparation of tea infusions

Tea infusions of all samples described above (see Table 1) were prepared according to manufacturerís instructions. Briefly, 1 portion of dry herb (1.5 g) and 250 mL of boiling water was used for tea infusion preparation in cases of samples 1 and 2. The samples 3 and 4 were processed using one tea bag (1.5 or 3.0 g) and boiling water (250 or 500 mL). Antimicrobial testing was performed after leaching for 10–15 min of all sample type.

2.4.3. Extraction of volatiles from distilled aromatic water and tea infusion

Volatile composition of distilled aromatic water and tea infusion were determined by the use of headspace-solid phase microextraction (HS-SPME). 10 mL of distilled aromatic water was transferred into 20 mL headspace vial. The vial was then closed by a cap with Teflon septum. Sample was incubated at 100 °C for 20 min to obtain steady-state extraction conditions. The extraction was performed using a 100 μm PDMS fiber at temperatures decreasing from 100 °C to 30 °C within 75 min. After that, volatile compounds were released from the fiber in the GC injector port, set up at 200 °C.

2.5. GC–MS analysis

A gas chromatograph mass spectrometer GC2010-QP2010 Plus (Shimadzu, Kyoto, Japan) and PAL-Combi auto-sampler (CTC Analytics AG, Zwingen, Switzerland) was used for analysis. A capillary column SLB–5 ms with length 30 m, 0.25 mm inner diameter and 0.25 μm film thickness (Supelco, Bellefonte, PA, USA) was used for separation in all analysis. Helium 5.0 (Linde Gas a.s., Prague, Czech Republic) at a constant linear velocity of 30 cm/s was used as the carrier gas. The injector and the interface temperature were maintained at 200 °C. Injections of essential oils have been performed in split mode 1:5. The column temperature has been programmed as follows: the initial temperature was 40 °C (1 min) then increased at a rate of 2 °C/min up to 200 °C (20 min). The mass spectrometer was operated in the full scan mode over a mass range of *m*/*z* 33–450 using the electron ionization (70 eV). The mixture of *n*-alkanes was injected using the above mentioned temperature program in order to calculate the retention indices.

2.6. GC-FID analysis

Shimadzu GC 2010 gas chromatograph equipped with flame ionization detector (FID) was used for GC-FID analysis of essential oils. The detector temperature was set on 220 °C. Conditions of measurements, including the column type and column temperature, the injector temperature, split ratio, carrier gas and the linear velocity, were set like those of GC–MS analysis.

2.7. Antimicrobial assay (antibacterial activity)

A microdilution assay using Mueller-Hinton broth (MHB, Himedia, Mumbai, India) was used to determine the antimicrobial