Supporting Information

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Chemical Composition and Biological Activity of Essential Oils of Sempervivum brevipilum Muirhead

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S1: Experimental

Plant Material

Sempervivum brevipilum Muirhead. was collected from Kalebaşı plateau in Koyulhisar, Sivas-Turkey (at heights of ~1750m) in July 25, 2011. The plant was authenticated by Prof. K. Coşkunçelebi [1, 2]. Voucher specimen was deposited in the Herbarium of the Department of Biology, KATUB (Coşkunçelebi 731), Karadeniz Technical University, Turkey.

Isolation of the essential oils

The fresh plant materials were separated into flower, leaf, and stem parts and they were grounded into small pieces. The essential oils from fresh aerial parts (~240 g, each) of *S. brevipilum* were isolated by hydrodistillation in a modified Clevenger-type apparatus [3-10] with cooling bath (-15 ^{o}C) system (4h) (yields: 0.015%, 0.010%, and 0.013% (w/w), respectively). The obtained oils were extracted with HPLC grade n-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at 4-6 ºC in a sealed brown vial.

Gas chromatography (GC)

The capillary GC-FID analysis was performed using an Agilent-5973 Network System, equipped with a FID (supplied with air and hydrogen of high purity) and a split inlet. The chromatographic column used for the analysis was HP-5 capillary column (30 m x 0.32 mm i.d., film thickness 0.25 μm). Helium was used as carrier gas, at a flow rate of 1 ml/min. The injections were performed in splitless mode at 230 °C. Two μl essential oil solution in hexane (HPLC grade) 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. The identity of each compound was supported by comparing their retention indices (RI) with published values [3-12]. The sample was analyzed twice and the percentage composition of oil was computed from the GC peak areas without using correction factors.

Gas chromatography-mass spectrometry (GC/MS)

GC-MS analysis was performed using an Agilent-5973 Network System. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column used for the analysis was HP-5 capillary column (30 m x 0.32 mm i.d., film thickness 0.25 mm). Helium was used as carrier gas, at a flow rate of 1 ml/min. The injections were performed in splitless mode at 230 °C. Two ml essential oil solution in hexane (HPLC grade) 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.

Identification of components

Retention indices of all the components were determined by Kovats method using *n*-alkanes (C_6-C_{32}) as standards. Identification of individual components was made by comparison of their retention times with those of available analytical standards (α-pinene, camphene, β-pinene, limonene, γ-terpinene, nonane, eicosane, heneicosane, docosane, tricosane, tetracosane, and pentacosane), and by computer search, computer search, matching mass spectral data with those held in NIST (Version 2.0 a) and Wiley (Wiley7n.1) library of mass spectra and literature comparison [3-14].

Antimicrobial activity assessment

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* (ATCC 25922), *Yersinia pseudotuberculosis* (ATCC911), *Pseudomonas aeruginosa* (ATCC43288), *Enterococcus faecalis* (ATCC29212), *Staphylococcus aureus* (ATCC25923), *Bacillus cereus* 709 Roma, *Mycobacterium smegmatis* (ATCC607), *Saccharomyces cerevisiae* (RSKK251) and *Candida albicans* (ATCC60193). All essential oils of flower, leaf and stem were weighed and dissolved in hexane to prepare extract stock solution of 10.100, 21.880, and 10.800 µg/mL, respectively. The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration (MIC) values (µg/mL) were determined [15]. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. Brain Heart Infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis* [16]. The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10µg), Streptomisin (10µg) and fluconazole (5µg) were used as standard antibacterial and antifungal drugs, respectively. Hexane with dilution of 1:10 was used as solvent control.

S2: References

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