SUPPLEMENTARY MATERIAL

Antifungal and phytotoxic activity of essential oil from root of *Senecio amplexicaulis* Kunth. (Asteraceae) growing wild in High Altitude Himalayan Region

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Abstract

This work was aimed to evaluate the essential oil from root of medicinally important plant *Senecio amplexicaulis* for chemical composition, antifungal and phytotoxic activity. The chemical composition analysed by GC / GC-MS showed presence of monoterpene hydrocarbons in high percentage with marker compounds as α -phellandrene (48.57%), o-cymene (16.80%) and β -ocimene (7.61%). The essential oil exhibited significant antifungal activity against five phytopathogenic fungi, *Sclerotium rolfsii*, *Macrophoma phaseolina*, *Rhizoctonia solani*, *Pythium debaryanum* and *Fusarium oxysporum*. The oil demonstrated remarkable phytotoxic activity in tested concentration and significant reduction in seed germination percentage of *Phalaris minor* and *Triticum aestivum* at higher concentrations. The roots essential oil showed high yield for one of its marker compound (α -phellandrene) which makes it important natural source of this compound.

Keywords: *Senecio amplexicaulis*; essential oil; α -phellandrene; antifungal; phytotoxic activity.

3. Experimental Section

3.1 Plant material

Root of *S. amplexicaulis* Kunth. was collected from Chamoli Garhwal (North-West Himalayas), Uttarakhand, India at an altitude of 3600 m. The plant was identified and deposited at Botanical Survey of India, Dehradun, India with voucher no. 42099.

3.2 Procurement of the Chemicals

Potato dextrose agar (PDA), nutrient broth and nutrient agar were procured from Hi-Media Pvt. Ltd. (India). Tween-20 and other chemicals/solvents were obtained from Merck (India).

3.3 Essential oil Extraction

The chopped roots were air dried at room temperature and then were subjected to hydrodistillation for 3h using a Clevenger apparatus. The extracted light pale yellow essential oil was dried over anhydrous sodium sulphate to remove traces of water. The oil was stored in sealed vial at low temperature ($< 0^{\circ}\text{C}$) until used for analysis.

3.4 GC-FID and GC-MS analysis

Essential oil was analyzed for chemical composition on an Agilent capillary GC-FID (7890A) and GC-MS (5975) mass instrument equipped with a HP-5 column (30m $\times$ 0.25mm, film thickness 0.25 μm). Helium was used as a carrier gas (1.0 mL/min). Injection volume was 0.5 μL essential oil (10%) in hexane. The initial oven temperature was maintained at 60 $^{\circ}\text{C}$ and programmed to increase at 3 $^{\circ}\text{C min}^{-1}$ to 250 $^{\circ}\text{C}$ (held constant for 1min). The injector temperature was maintained at 260 $^{\circ}\text{C}$. The mass spectra were recorded with electron energy of 70eV over a range of 50 to 550 amu and ion source temperature at 230 $^{\circ}\text{C}$. In order to obtain the same elution order with GC-MS, simultaneous injection was done using the same column and appropriate operational conditions. The same column and analysis conditions were applied for both GC-FID and GC-MS.

Identification of the essential oil constituents was carried out by comparison of their relative retention times with those of authentic samples or by comparing their relative retention index (RI) to series of n-alkanes, MS Library search (NIST & Wiley) and/or by comparison with the literature data (Adams, 1995).

3.5 *In vitro* Antifungal activity

The antifungal activity of the compounds was evaluated *in Vitro* by poisoned food technique against against five phytopathogenic fungi, *Sclerotium rolfii* (ITCC 6263), *Macrophomina phaseolina* (ITCC 6267), *Rhizoctonia solani* (ITCC 4502), *Pythium debaryanum* (ITCC 95) and *Fusarium oxysporum* (ITCC 6246) (Shukla et al, 2012). Briefly, the ready-made PDA medium (39 g) was suspended in 1000 ml of distilled water and heated to boiling to completely dissolve the medium. The stock solutions of essential oil were prepared by dissolving sample (130 mg) in 2 ml of Tween 20-Water solution (0.1% v/v). Appropriate quantity of test sample in Tween 20 (0.1% v/v) was added to molten PDA medium (65 mL) in order to get the desired concentration of 250, 125 and 62.5 $\mu\text{g/mL}$ of oil. A mycelia disk of 5 mm in diameter cut from the 7 day old culture was inoculated in the center of each PDA plate and then inoculated in the dark at $27\pm 1^{\circ}\text{C}$ for seven days. PDA plates treated with Tween 20 (0.1% v/v) without essential oil was used as negative control. In addition, PDA plates treated with hexaconazole, a standard reference fungicide were used as a positive control. Experiments were repeated in triplicate, under aseptic condition in a laminar flow chamber.

3.7 Seed germination and seedling growth experiments

Seeds of *Phalaris minor* and *Triticum aestivum* were used in herbicidal assays. To avoid possible inhibition of germination due to fungal or bacterial toxins, seeds were surface sterilized with 15% sodium hypochlorite solution for 20 min, then rinsed with abundant distilled water. Germination was carried out on Petri dishes where seeds were placed on double-layered Whatman No. 1 filter paper moistened with different concentrations (0, 125, 250 and 500 µg/mL) of essential oil in a 1% solution of Tween 20 (Tworkoski 2002). Cultures were incubated under controlled conditions (25⁰C, 70% of relative humidity and 16/8 photoperiod). The Petri dishes were closed and sealed with adhesive tape to prevent the volatile oil from escaping. The numbers of germinated seeds were counted and seedling lengths were measured after seven days. The assays were arranged in a completely randomized design with three replications (20 seeds each) including controls.

References:

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