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Diplotaxis tenuifolia (L.) DC., a source of a potentially antifungal essential oil containing nitrile

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1. Subject and source

Diplotaxis tenuifolia (L.) DC., commonly known in Argentina as ''Flor amarilla'', belongs to the Brassicaceae family. Recent ethnopharmacobotanical studies, conducted in Central and Southern Italy, refer to *D. tenuifolia* as a traditionally used plant with therapeutic properties (Guarrera, 2003; Pieroni et al., 2004; Leporatti and Corradi, 2001) and consumed as salads (Scherrer et al., 2005; Guarrera, 2003). This perennial herb, native from Europe and Asia, was introduced in our country as a good melliferous plant at the beginning of the 20th century. Nowadays, it is classified as an invasive weed, noxious for agriculture.

Aerial parts of *D. tenuifolia* were collected in March 2004, at Bahía Blanca city, Buenos Aires province, Argentina. The identification of the plant was made by Lic. M.G. Murray and a voucher specimen (MGM93) is kept in the ''Herbario del Departamento de Biología, Bioquímica y Farmacia – Universidad Nacional del Sur (BBB)''.

2. Previous work

The nitrile 5-methylthiopentanenitrile has been detected as a minor constituent (5%) of the volatile fraction of *Eruca sativa* (Brassicaceae) leaves by headspace analysis (Jirovetz et al., 2002). Nitriles, thiocyanates and isothiocyanates are degradation products of glucosinolates, a large class of compounds produced by Brassicaceae (Fenwick et al., 1983). Several reports can be found in the literature about the antifungal activity of these compounds on postharvest pathogens (Mari et al., 1993, 1996, 2002).

3. Present study

The plant material (720 g) consisting of fresh picked aerial parts (leaves, stems and flowers) was subjected to hydrodistillation using a Clevenger type apparatus for 4 h. After this time, the yellowish oil obtained (0.574 g) was dried

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over anhydrous sodium sulfate, and analyzed by GC–MS. This analysis was carried out with Hewlett-Packard 5972A mass spectrometer equipped with a capillary column (HP-5, 30 m × 0.25 mm, 0.25 μm film thickness) using helium as carrier gas (1 ml/min). GC oven temperature was held at 50 °C for 5 min, programmed at 5 °C/min to 250 °C, then held at this temperature for 15 min. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 50 to 500 amu. The temperature of the injection block was 250 °C. The major constituent (91%) of the oil was identified as 5-methylthiopentanenitrile by comparison of its mass spectra [*m/z* 129 (52.6%), 114 (7.9%), 82 (39.5%), 61 (100%), 55 (20.4%), 54 (14.5%)] with that stored in the MS database (NBS75K.L MS DATA). The identity of this compound was confirmed by its ^{1}H and ^{13}C NMR spectra, recorded in $CDCl_3$ on a Bruker ARX 300 spectrometer at 300 MHz and 75 MHz, respectively. The ^{1}H NMR spectrum showed signals at δ 2.44 (t, 2H), 2.29 (t, 2H), 2.00 (s, 3H) and 1.67 (m, 4H). The ^{13}C NMR spectrum displayed signals at δ 119.1 (s), 32.8 (t), 27.4 (t), 23.9 (t), 16.4 (t) and 15.0 (c). The absorption due to the nitrile function was detected at 2244 cm^{-1} in the IR spectra recorded on a Nicolet NEXUS FT spectrometer. Other absorptions registered were: 2928, 2862, 1456, 1270, 1134, 1076 and 746 cm^{-1}.

The antifungal activity of the distilled oil was evaluated by the paper disk-agar diffusion method against *Penicillium digitatum*, *Penicillium funiculosum*, *Fusarium oxysporum*, *Phytophthora infestans* and *Rhizopus* sp. Paper disks (diameter: 5 mm) impregnated with 10 μl of oil dilutions of known concentrations (from 10 mg/ml to 100 mg/ml in CH_2Cl_2) were applied over the previously inoculated test plates prepared with potato dextrose agar medium. A disk negative control (10 μl CH_2Cl_2) was included. Amphotericin B and metalaxyl (for *P. infestans*) were included as positive controls. After 3 days of incubation at 27 °C (7 days for *P. infestans*) the MIC (mg/disk) were determined as the lowest concentrations causing mycelium-free zones. All the assays were carried out in triplicate.

The product obtained by steam distillation from fresh leaves, stems and flowers of *D. tenuifolia* show no activity against *F. oxysporum* and *Rhizopus* sp. at the tested concentrations (MIC > 1 mg/disk in both cases). On the other hand, a moderate antifungal activity was observed against *P. infestans* (MIC = 1 mg/disk), *P. digitatum* (MIC = 0.1 mg/disk) and *P. funiculosum* (MIC = 0.1 mg/disk).

4. Chemotaxonomic and ecological significance

The detection of 5-methylthiopentanenitrile provides evidence for the occurrence of 4-methylthiobutylglucosinolate in *D. tenuifolia*. This observation is also supported by the results reported by Cole (1976) for the autolytic hydrolysis products obtained for *D. tenuifolia* where 4-methylthiobutyl isothiocyanate was the only aglucone found in the volatiles of this species as well as in *Diplotaxis viminea* (L.) DC. and the major one found in *Diplotaxis erucoides* (L.) DC. Methylthiobutyl isothiocyanate had been previously reported from *D. tenuifolia* by Kjaer (1960). Also from this species has been reported the presence of the acid dimethylsulfonium-5-pentanoic in the flowers (Larher and Hamelin, 1979).

We found that the use of this compound, obtained from *D. tenuifolia* with minimum cost, against phytopathogenic or postharvest fungus appears promising as an economically viable alternative for the exploitation of a widely distributed weed.

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