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Comparative studies on the antimicrobial and cytotoxic activities of *Tanacetum vulgare* L. essential oil and methanol extracts

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ABSTRACT

Chemical composition of essential oil (EO) and methanol extracts (MEs) from different parts of *Tanacetum vulgare* L. plant was analyzed and investigated for potential biological activities and correlated with the main constituents detected in EO and MEs.

The EO was characterized by a high content of oxygenated monoterpenes with *trans*-chrysanthenyl acetate as major compound. All MEs were characterized by neochlorogenic, 3,5-*O*-dicaffeoylquinic and caffeoylquinic acids. High phenolic content in MEs correlated to high antioxidant capacity, especially for roots. Tansy EO showed strong activity against the most of tested fungi and was efficient as bifonazole and ketoconazole, while the most sensitive bacteria were Gram-negative *E. coli* and *E. cloacae*. All MEs showed fungistatic and fungicidal effects against all of the eight tested fungi, but the antimicrobial activity was higher against Gram-positive bacteria. Additionally, shown for the first time, MEs of leaves and flowers exhibited a strong antiproliferative effect on human cervical adenocarcinoma (HeLa) cells, causing cell shrinkage and detachment.

In the present study, the Tansy extracts and essential oil with low thujone content can provide a very promising and effective alternative in the field of antimicrobial applications and food preservation. The Tansy MEs possess a high antioxidant potential with phenolic acids being a major radical scavenging contributor with the proved antiproliferative activity to HeLa cells.

1. Introduction

Tanacetum vulgare L. (Asteraceae/Compositae, syn. *Chrysanthemum vulgare* L.), is an aromatic perennial plant, widely spread in the northern hemisphere growing as wild weed on waste ground, roadsides and close to water (Heywood, 1976). Its common name "Tansy" comes from the Greek word "athanasia" which means "immortality", which probably originated from the fact that its flowers do not wilt when dry. The coherence of Tansy with immortality makes these plants used for embalming in the Middle Ages (Haughton, 1978).

Plants from the genus *Tanacetum* are rich in essential oils (EOs) and have been used in traditional medicine from the ancient times. Tansy is conventionally used in balsams, cosmetics, dyes, insecticides, medicines, and preservatives (Grieve, 1984). Extracts from this plant are used extensively in modern medicine for treating rheumatism, ulcers, fever, and digestive disorders. The crude toxic drug Tanaceti flos has been used for years in some western pharmacopeias because of its vermifuge and emmenagogue properties (Evans, 1996). In Russian traditional medicine, an infusion of the flowers is used for wound healing, improving appetite and as analgesic (Zaurov et al., 2013). Tansy has been cultivated in gardens and used as a spice in human diets (Heywood, 1976; Grieve, 1984; Mitich, 1992; Mabey, 1996).

Over the years, numerous researches related to the beneficial properties of Tansy extracts and secondary metabolites have emerged. For example, it has been shown that chloroform, acetone and methanol extracts from Tansy leaves and/or flowers exhibited an anti-inflammatory activity (Mordujovich-Buschiazzo et al., 1996; Brown et al., 1997; Williams et al., 1999). Similarly, Lahlou et al. (2007) demonstrated that the water extract of Tansy leaves has a strong diuretic action and no renal toxicity or any other detrimental effects. The same research group (2008) showed vasodilatory properties of aqueous extract of Tansy and validated the empirical use of this plant as antihypertensive

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in Moroccan pharmacopeia. Ethanol-water extracts of Tansy leaves and flowers showed anticandidal activity, as well as, some degree of antibacterial activity (Holetz et al., 2002). Additionally, Xie et al. (2007) supported the concept of using Tansy polysaccharides as an immunotherapeutic adjuvant.

Chiasson et al. (2001) tested its EO against the spotted spider mites, *Tetranychus urticae* Koch, and demonstrated strong acaricidal properties. The activity against microbes and insects was dependent on the chemical composition of the essential oil (Schearer, 1984; Holopainen and Kauppinen, 1989). Certain components of the EOs are also of potential interest as aroma chemicals in perfumery (Lawrence, 1992).

Tansy populations show high variability in regards to the essential oil composition. More than 30 chemotypes have already been classified, according to the most dominant constituent in the oil (Nano et al., 1979; Gallino, 1988; Holopainen, 1989; Neszmélyi et al., 1992; Collin et al., 1993). Commercial oils of Tansy are mostly of the thujone type. Thujone is a bioactive compound with medicinal properties, but at high concentrations, it exhibits toxicity (Woolf, 1999; Sirisoma et al., 2001).

The aim of this study was to determine the chemical composition of essential oil (EO) and methanol extracts (MEs) from different parts of the Tansy plant and to evaluate their radical scavenging capacity as well as antibacterial, antifungal and antiproliferative efficacies. Considering thujone toxicity this study aimed to identify the most effective Tansy extract that might have a commercial value in the production of functional bioactive ingredient for food, pharmaceutical and agricultural use.

2. Materials and methods

2.1. Chemicals and reagents

Milli-Q water was generated by deionization (Millipore, Billerica, USA). Acetonitrile, ethanol, formic acid and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Gallic acid, (+)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich (Steinheim, Germany) and used for the total phenolic content and the free radical scavenging activity assays. Potassium ferricyanide (Sigma–Aldrich, Steinheim, Germany), trichloroacetic acid (Serva, Heidelberg, Germany) and iron (III) chloride (Merck, Darmstadt, Germany) were used for reducing power ability assay. Streptomycin (Polfa, Tarchomin, Poland), ampicillin (Panfarma, Belgrade, Serbia), penicillin (Hemopharm, Banja Luka, Bosnia and Hercegovina) bifonazole (Srbolek, Belgrade, Serbia), ketoconazole (Zorkapharma, Šabac, Serbia) and iodonitrotetrazolium chloride (INT) (Sigma–Aldrich, Steinheim, Germany), were used in antimicrobial and antiproliferative assays. Dimethyl sulfoxide (DMSO) was purchased from Duchefa (Haarlem, The Netherlands) and used as negative control in antimicrobial assays. L-glutamine, sulforhodamine B (SRB), RPMI 1640 medium, inactivated fetal calf serum (FCS), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), CDDP (cis-diamminedichloroplatinum (II)) (cisplatin) and acetic acid were purchased from Sigma-Aldrich (Steinheim, Germany) and used for antiproliferative assay.

2.2. Plant material

The study was conducted on the wild type of *T. vulgare* L. 1573, in the flowering stage collected from the Ada Huja locality, Belgrade, Serbia, in 2012. The plant has been authenticated by the authors, rev.: Dr. Goran Anačkov, dried and prepared as herbarium specimens to be deposited at the Herbarium of the Department of Biology and Ecology–BUNS Herbarium, Faculty of Natural Sciences, University of Novi Sad, voucher No. 2-2069.

The flower heads, leaves, stalks and roots were excised at the onset of flowering, air dried and stored at the room temperature in the dark until extraction.

2.3. Essential oil isolation

Air-dried aerial parts of Tansy were separated from wooden parts and placed to 3 h of hydrodistillation, using Clevenger-type apparatus according to the standard procedure (Ljaljević-Grbić et al., 2008). The obtained EO was stored in sealed dark vials, at 4 °C for further analyses.

2.4. Methanol extract preparation

For the preparation of Tansy methanol extracts from leaves, stalks, flowers and roots, 30 g of air-dried tissues were separately blended in an electric blender to fine powder and extracted with methanol (300 mL) in an ultrasonic bath for 20 min. After sonication, the extraction was continued by maceration of tissue for 48 h in the dark at room temperature. The extracts were filtered through Whatman filter paper No. 4 and supernatants were evaporated in vacuum evaporator (rotary evaporator Buchi R-210, Flawil, Switzerland) at 40 °C to dryness. The extracts were stored at the room temperature for further analyses.

2.5. Essential oil analysis by gas chromatography (GC/FID) and gas chromatography/mass spectrometry (GC/MS)

GC/FID analysis of EO was carried out on the Agilent 7890A Gas Chromatograph (Agilent Technologies, Wilmington, DE, USA), equipped with split-splitless injector and automatic liquid sampler (ALS), attached to HP-5 column (30 m × 0.32 mm, 0.25 μm film thickness) and fitted to flame ionization detector (FID). Carrier gas (H_2) flow rate was 1 mL/min, injector temperature was 250 °C, detector temperature 300 °C, while column temperature was linearly programmed from 40 to 260 °C (at rate of 4°/min), and held isothermally at 260 °C next 10 min. Solutions of EO in ethanol (~1%) were consecutively injected by ALS (1 μL, splitless mode). The area percent reports, obtained as a result of standard processing of chromatograms, were used as the base for quantification purposes.

Similar analytical methods were employed for GC/MS analysis, along with column HP-5MS (30 m × 0.25 mm, 0.25 μm film thickness), using HPG 1800C Series II GCD system (Hewlett-Packard, Palo Alto, CA, (USA). Instead of hydrogen, helium was used as carrier gas. Transfer line was heated at 260 °C. The mass spectra were acquired in EI mode (70 eV) in m/z range 40–450. Sample solutions were injected by ALS (1 μL, splitless mode).

The constituents were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1., National Institute of Standards and Technology- NIST, Standard Reference Data Program, Gaithersburg, MD, USA), compared to those from available literature and used as additional tool to approve MS findings (Adams, 2007).

2.6. Liquid chromatography/mass spectrometry (LC/MS) analyses of methanol extracts

For LC-DAD/ESI ToF MS analyses of MEs (c = 10 mg/mL) an 6210 Time-of-Flight LC–MS system (Agilent Technologies, Santa Clara, California, USA) was connected to an Agilent 1200 Series HPLC instrument (Agilent Technologies, Waldbronn, Germany), with a degasser, a binary pump, an autosampler, a column compartment equipped with a Zorbax Eclipse Plus C18 column (1.8 μm, 4.6 mm × 150 mm, Agilent Technologies) and a diode-array detector, *via* ESI interface. The mobile phase consisted of water containing 0.2% formic acid (A) and