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Agrorefinery of *Tanacetum vulgare* L. into valuable products and evaluation of their antioxidant properties and phytochemical composition

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ABSTRACT

Volatile oils constitute only a minor fraction in the essential oil (EO) bearing plants, therefore their distillation residues should be processed into other valuable products. *Tanacetum vulgare* investigated in this study yielded 0.52 mL 100 g^{-1} of EO containing 86% of β-thujone. Liquid hydrodistillation residue was dried yielding water extract (WE), while solid residue and the whole material were extracted with acetone obtaining acetone oleoresin (AO) and deodorized acetone extract (DAE). Antioxidant properties of tansy extracts were evaluated by different *in vitro* and *in situ* methods. Total phenolic content in the extracts was in the range of 34.0–142.3 mg gallic acid equivalents/g, effective radical scavenging concentrations (EC_{50}) were 1.33–13.19 mg/mL (DPPH*), 1.40–3.49 mg/mL (ABTS**), while ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) values were, 157.3–546.2 and 4285.4–11947.5 μmol trolox equivalents/g, respectively. WE was the strongest antioxidant *in vitro* assays and in stabilizing mayonnaise, while AO additives were the most effective in stabilizing rapeseed oil, and the PFs obtained were 2.46 and 2.83, respectively. The on-line HPLC–UV–DPPH* analysis showed that mono and dicaffeoylquinic acids were the major contributors to the antioxidant capacity of WE. Fifteen phenolic constituents (mainly derivatives of luteolin, apigenin and chlorogenic acid) were identified in *T. vulgare* extracts by UPLC/Q-TOF/MS2. In general, the study demonstrated strong antioxidant potential of *T. vulgare* and results obtained may assist in selecting the most valuable tansy extracts for the production of bioactive ingredients for foods, medicinal and biotechnological or agricultural applications.

1. Introduction

Development of natural products has become an important issue in many production areas, including foods, cosmetics, pharmaceuticals, organic agriculture, sanitary and hygienic goods, etc. The kingdom of Plantae comprising more 300 thousands species is the main source of such products. However a great number of plant species remains underinvestigated, while comprehensive data on their properties and chemical composition as well as processing technologies may lead to the development of valuable industrial crops and products for various applications. This study is focusing on one of such species, tansy (*Tanacetum vulgare* L., Asteraceae), which is an aromatic plant spread mainly in the northern hemisphere in Europe, Asia, and North America. Due to the multifunctional properties of bioactive compounds accumulated in tansy, it has been traditionally used as a food spice, in cosmetics, and as a herbal remedy. Herbal preparations of tansy have been used against intestinal worms, kidney disease, respiratory infections and as an abortivum (Dragland et al., 2005). Occasionally, it has been grown in gardens and used in omeletts, salads, cakes and spice mixtures (Keskitalo et al., 2001). The leaves of tansy can be used as a spice instead of cinnamon and nutmeg, whereas due to a strong scent derived from the EO, the plants have traditionally been used as a repellent and deterrent against flies and other insects (Dragland et al., 2005). EOs and/or extracts of tansy were reported to exhibit anti-inflammatory, antibactericidal, antifungicidal, and insects repellent activities; which was dependent on the chemical

Abbreviations: EO, Essential oil; WE, water extract; AO, acetone oleoresin; DAE, deodorized acetone extract; EDW, extract dry weight; pdw, plant dry weight; RSC, radical scavenging capacity; SET, single electron transfer; HAT, hydrogen atom transfer; TPC, total phenolic content; GA, gallic acid; TEAC, trolox equivalents antioxidant capacity; RO, rapeseed oil; MA, mayonnaise; PF, protection factor; IP, induction period; KI, Kováts retention indices.

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composition of the EO (Keskitalo et al., 2001). The components in the tansy EO are also of interest as aroma chemicals in perfumery (Lawrence, 1992).

Chemical composition of tansy EO has been reported in numerous studies and 23 chemotypes were determined according to the most dominant constituents (Lawrence, 2000), however, despite the great EO variability, the thujone, camphor, cineole, chrysanthenyl, artemisia, and umbellulone types are the most common in Europe (Rohloff et al., 2004; Dragland et al., 2005; Forsén and Von Schantz, 1974; Holopainen and Kauppinen, 1989; Keskitalo et al., 2001; Mockute and Judzentiene, 2003, 2004; Judzentiene and Mockute, 2005). *T. vulgare* is widely distributed in Lithuania as the only *Tanacetum* genus species; two varieties, var. *vulgare* and var. *crispum* are found (Lekavičius, 1980). Tansy antioxidants have been studied rather scarcely (Dragland et al., 2003; Juan-Badaturuge et al., 2009). For instance, its acetone extract was reported as inhibiting rapeseed oil oxidation, particularly at higher concentrations (Bandonienė et al., 2000).

Production of EOs results in a very high content of residues, which usually remain unused after distillation. To increase the efficiency of processing of aromatic plants, such residues should be carefully investigated for assessing the possibilities of their further conversion into valuable products. Therefore, more systematic studies using biorefinery (sometimes called agrorefinery) concept are required in order to expand the possibilities of a wider application of *T. vulgare* for foods, nutraceuticals, cosmetics, medicinal and other purposes.

This study was aimed at evaluating phytochemical and antioxidant characteristics of *T. vulgare* grown in Lithuania by using a more systematic agrorefinery concept, which has not been previously applied to this plant. Therefore, the main innovation aspects of this study are focused on rational processing of plant raw material, antioxidant potential and nonvolatile phytochemicals. The main objectives were to determine the chemical composition of EO, to evaluate oxygen radical absorbance and radical scavenging capacities, total content of polyphenolics and to identify the main antioxidant constituents in different *T. vulgare* extracts, which might have commercial value in the production of functional bioactive ingredients for foods, medicinal, biotechnological, and agricultural uses. Additionally, the stability of rapeseed oil and mayonnaise with addition of tansy extracts was tested by instrumental methods.

2. Materials and methods

2.1. Plant material, isolation of EO and preparation of plant extracts

Tansy (*Tanacetum vulgare* L.) was cultivated in Kaunas Botanical Garden of the Vytautas Magnus University (Lithuania). Harvested herbs were dried at 40 °C in the dark. The EO was isolated from dried herb in a Clevenger-type apparatus during 2 hours and analysed by GC–MS. The residues after distillation were separated into liquid and solid fractions by filtration. The liquid fraction was freeze-dried resulting in dry water extract (WE); the solid fraction (deodorised herb) was dried at 40 °C. Acetone oleoresin (AO) and deodorised acetone extract (DAE) were produced by extracting whole and deodorised plant material with acetone. All extractions were carried out in three steps at room temperature for 3 h; solid material and solvent ratio for the whole and deodorised plants was 1–17.7 (w/v) and 1–24.3 (w/v) respectively. The solids after each extraction were separated from the liquid by filtration and dried at 40 °C in a drying oven. The AO and DAE extracts were concentrated in a Büchi rotary vacuum evaporator (Flavil, Switzerland) at ~45 °C. The yields of the tansy plant extracts obtained were as follows: WE (22.96 ± 0.50%) > AO (4.26 ± 0.17%) > DAE (2.15 ± 0.01%). The results are expressed in extract dry weight (edw) and/or in plant dry weight (pdw).

2.2. Chemicals

2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH*, 95%), gallic acid, tetramethylchromane-2-carboxylic acid (Trolox 97%), anhydrous sodium carbonate, 2,20-azobis (2-amidinopropane) dihydrochloride (AAPH) were purchased from Sigma–Aldrich Chemie (Steinheim, Germany); 2,4,6-tripyridyl-s-triazine (TPTZ) and fluorescein (FL) was from Fluka Chemicals (Steinheim, Germany); aluminium trichloride hydrate and sodium acetate from Reachim (Riga, Latvia); 2.0 M Folin–Ciocalteu phenol reagent, 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diamonium salt (ABTS), NaCl, KCl, Na_2HPO_4 and $K_2S_2O_8$ were from Merck (Darmstadt, Germany); KH_2PO_4 was from Jansen Chimica (Beerse, Belgium); pentane (>99%), acetone, methanol (99.8%) and acetic acid (98%) was from Lachema (Brno, Czech Republic), the reference substance (+)-catechin (98.3%) was purchased from Chromadex (California, USA) and chlorogenic acid (3-*O*-caffeoylquinic acid, 97%) from Roth (Karlsruhe, Germany).

A mixture of C_8-C_{32} *n*-alkanes (Sigma Chemical Co., St. Louis, MO) was used to determine Kováts retention indices (KI). The following reference compounds (95–99% purity) for the identification of tansy EO volatiles were purchased from Fluka and Sigma–Aldrich: α-thujene, α-pinene, camphene, sabinene, γ-terpinene, d-limonene, 1,8-cineole, terpinolene, β-thujone, camphor, borneol, terpinen-4-ol, α-terpineol, bornyl acetate, β-caryophyllene, α-humulene, δ-cadinene, spathulenol, caryophyllene oxide.

2.3. Gas chromatography (GC)

The EO diluted in pentane (5 μL in 1 mL; 0.5% v/v) was analyzed on a *Fisons 8000* series gas chromatograph (Fisons Instruments Inc., Rodano MI, Italy) equipped with a flame ionization detector (FID) and a DB–5 fused silica capillary column (polydimethylsiloxane, 5% phenyl, 50 m length, 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA). The carrier gas was helium at a linear flow velocity of 32.7 cm s^{-1} at 50 °C which was equivalent to a 2.35 mL min^{-1} volumetric flow; the detector's temperature was 320 °C, the oven temperature was programmed from 50 °C (2 min) to 280 °C (10 min) at the rate of 5 °C min^{-1}. A split/splitless injector was used at 250 °C in a 1:10 split mode; the injection volume was 1 μL. The content of the eluted compounds was calculated on a DP800 integrator and expressed as a GC peak area percentage. The mean values were calculated from 4 injections.

2.4. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were performed using a Perkin Elmer *Clarus 500* gas chromatograph coupled to a Perkin Elmer *Clarus 500* series mass selective detector (Perkin Elmer Instruments, Shelton, USA) in the electron impact ionization mode at 70 eV, the mass range was *m/z* 29–550. Volatile compounds were separated using an Elite–5MS capillary column (dimethylpolysiloxane, 5% diphenyl, 30 m length, 0.25 mm i.d., 0.25 μm film thickness, Perkin Elmer Instruments, Shelton, USA). The oven temperature was programmed as described above. Carrier gas, helium, was set to a linear velocity of 36.2 cm/s at 50 °C or 1.0 mL/min volumetric flow. Split ratio was 1:20, injector's temperature 250 °C.

The identification was based on KI obtained on nonpolar DB–5 (Adams, 2009) and by comparing mass spectra with the data present in NIST (vers.1.7), NBS 75 K/WILEY 275, EPA/NIH mass spectral libraries and literature sources (Adams, 2009). Additionally, the