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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

acetonitrile (B). A gradient program was used as follows: 0–30 min 10–25% B, 30–40 min, 25–55% B, 40–50 min, 55–100% B, 50–55 min, 100% B, 55–56 min, 100–10% B, and 56–61 min, 10% B. The flow rate of mobile phase was 0.8 mL/min, the column temperature was 40 °C and the injection volume was 2 μL. Spectral data from all the peaks were accumulated in the range of 190–650 nm and chromatograms were recorded at 280 nm. Full scan mass spectra were measured between 100 and 2500 *m/z* in negative ion (preferred) mode. Nitrogen was used as nebulizing gas at 45 psi, 350 °C and at a flow rate of 12 L/min. The mass spectrometric conditions were: negative ESI ionization mode, capillary voltage 4000 V, fragmentor voltage 70 V, skimmer voltage 60 V, OCT RF voltage 250 V. Eluted compounds were detected as $[M-H]^-$, $[M+HCO_2]^-$, $[2M-H]^-$ or $[M-2H]^{2-}$ signals using these parameters. The MassHunter Workstation software was used for data acquisition and processing. Compounds were characterized by their retention times (R_t), mass spectra and UV spectra.

2.7. Total phenolic content (TPC) and antioxidant activities of MEs

2.7.1. Total phenolic content (TPC)

The total phenolic content was estimated by Folin–Ciocalteu method as described by Singleton and Rossi (1965), with slight modifications. To 100 μL of each extract (0.5 mg/mL), 500 μL of Folin–Ciocalteu reagent (previously tenfold diluted with distilled water) was added and mixed. After 5 min, 400 μL of sodium carbonate (7.5 g/mL) was added, and mixture was incubated at room temperature in the dark for 2 h. The absorbance of samples was measured at 765 nm using Agilent 8453 UV–Visible spectrophotometer. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight of plant extract (mg GAE/g DW). Triplicate measurements were taken independently and mean values were calculated.

2.7.2. Radical scavenging activity (RSA)

The free radical scavenging activity of the extracts on the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was carried out according to the procedure described previously with some modifications (Brand-Williams et al., 1995). The antiradical capacity of samples was evaluated using a dilutions series (0.06–1 mg/mL), in order to obtain a large spectrum of sample concentrations. Extracts were re-dissolved in methanol (500 μL) and mixed with 500 μL of 150 μM methanol solution of DPPH. The obtained mixture (1 mL) was shaken vigorously and held for 20 min at room temperature in the dark. Thereafter, the absorbance of the bleaching of purple colored methanol solution of DPPH in samples was measured at 517 nm. All of the analyses were performed in triplicates, using Trolox, (+)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, a synthetic analog of vitamin E as a positive control. The percentage of inhibition was calculated using equation

$$\%\ \text{Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is absorbance of the control solution (containing only DPPH) and A_1 is the absorbance of the samples. The IC_{50} values (defined as the concentration of test compound required for 50% inhibition of free radicals) were calculated by linear regression of plots where the abscissa represented the concentration of tested samples and ordinate the average percent of inhibition activity from the three separate tests.

2.7.3. Reducing power ability (RPA)

The reducing power of tested MEs was estimated according to the method described by Xue et al. (2011) with several modifications. A dilution series of Trolox and methanol extracts (0.1–1 mg/mL) were mixed with phosphate buffer (0.2 mM, pH 7) and potassium ferricyanide (10 mg/mL). After incubation at 50 °C for 20 min, trichloroacetic acid (100 mg/mL) was added and the mixture was centrifuged at 2000 ×*g* for 10 min. The supernatant was mixed with distilled water and $FeCl_3$ (1 mg/mL) and the mixture was left at the room temperature for 10 min. The absorbance was measured by UV–Visible spectrophotometer at 700 nm. The reducing power assay used transformation of Fe^{3+} ion to Fe^{2+} ion to indicate the electron-donating capacity of bioactive compounds in tested extracts. The presence of reducers (*i.e.* antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form and measuring the formation of Perl's Prussian blue at 700 nm can display the Fe^{2+} concentration. The increased absorbance indicated increased reducing power. Triplicate measurements were taken independently and the mean values were calculated.

2.8. Antimicrobial activity of EO and MEs

2.8.1. Antibacterial activity

The following Gram-negative bacteria: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterobacter cloacae* (ATCC 35030), and Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), and *Listeria monocytogenes* (NCTC 7973) were used in order to estimate the potential antibacterial activity of Tansy EO and MEs. The microorganisms were obtained from the Mycological Laboratory, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia.

The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by the microdilution method (Espinel-Ingroff, 2001). Briefly, fresh overnight culture of bacteria was adjusted by the spectrophotometer to a concentration of 1×10^5 CFU/mL. The requested CFU/mL corresponded to a bacterial suspension determined in a spectrophotometer at 625 nm. Dilutions of inocula were cultured on solid medium to verify the absence of contamination and check the validity of the inoculum. Different solvent dilutions of methanol extracts/oil were carried out over the wells containing 100 μL of Tryptic Soy Broth (TSB) and afterwards, 10 μL of inoculum was added to all the wells. The microplates were incubated for 24 h at 37 °C. The MIC of each extract was detected following the addition of 40 μL of iodonitrotetrazolium chloride (INT) (0.2 mg/mL) and incubation at 37 °C for 30 min. The lowest concentration that produced a significant inhibition (around 50%) of the growth of the bacteria in comparison with the positive control was identified as the MIC. The minimum inhibitory concentrations (MICs) obtained from the susceptibility testing of various bacteria to tested extract/fraction were determined also by a colorimetric microbial viability assay based on reduction of INT color and compared with positive control for each bacterial strains (CLSI, 2009; Tsukatani et al., 2012). MBC was determined by serial sub-cultivation of 10 μL into microplates containing 100 μL of TSB. The lowest concentration that shows no growth after this sub-culturing was read as the MBC. Standard drugs, namely streptomycin and ampicillin were used as positive controls. Dimethyl sulfoxide (DMSO) (5%) was used as negative control.

2.8.2. Antifungal activity

For the antifungal bioassays, the following microfungi were used: *Aspergillus fumigatus* (1022), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730), *Aspergillus niger* (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Penicillium verrucosum* var. *cyclopium* (food isolate) and *Trichoderma viride* (IAM 5061). The organisms were obtained from the Mycological Laboratory, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia. The micromycetes were maintained on malt agar (MA) and the cultures were stored at +4 °C and subcultured once a month.

The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (*v*/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μL/well. The inocula were stored at +4 °C for further use. Dilutions of the inocula were cultured on solid MA

to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique using 96-well microtitre plates. The methanol extracts/oil were dissolved in 5% solution of DMSO and added to broth malt medium with fungal inoculum. The microplates were incubated for 72 h at 28 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MIC. The minimum fungicidal concentrations (MFCs) were determined by serial subcultivation of 2 μL in microtitre plates containing 100 μL of malt broth per well and further incubation for 72 h at 28 °C. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum (Espinel-Ingroff, 2001). Bifonazole and ketokonazole were used as positive controls. DMSO (5%) was used as negative control.

2.9. Antiproliferative activity of EO and MEs

2.9.1. Cell culture

Human cervical adenocarcinoma cells (HeLa) and human fetal lung fibroblast cells (MRC-5) were maintained in the RPMI 1640 medium. RPMI media were supplemented with 10% heat inactivated fetal calf serum (FCS), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES, 25 mM), penicillin (100 units/mL), streptomycin (200 μg/mL), and L-glutamine (3 mM). Cells were maintained as a monolayer culture in tissue culture flasks (Thermo Scientific Nunc™) in an incubator at 37 °C, in a humidified atmosphere composed of 5% CO_2.

2.9.2. SRB cell antiproliferative assay

Antiproliferative activity of tested EO and MEs was determined using sulforhodamine B colorimetric assay (Perez et al., 1993; Papazisis et al., 1997). Cells were seeded into 96-well cell culture plates (Thermo Scientific Nunc™), at a cell density of 4000 c/w (HeLa) or 7000 c/w (MRC-5), in 100 μL of culture medium. After 24 h of growth, cells were exposed to the serial dilutions of the tested EO and MEs (50 μL volume), or complete culture medium (control wells). Serial dilutions were made in culture medium so that the final concentration of ethanol per well was less than 0.1% (*v*/v). Each concentration of EO of MEs was tested in triplicates. The SRB assay was carried out according to modified protocol as previously described (Papazisis et al., 1997). In brief, culture medium was discarded prior to fixation with 50 μL of 10% cold (4 °C) trichloroacetic acid (TCA). Microplates were washed five times, with deionized water and left to dry at room temperature for at least 24 h. Subsequently, 50 μL of SRB solution (0.4% (*w*/*v*) in 1% (*v*/v) acetic acid) was added to each well, and left at room temperature for 20 min. SRB was removed and the plates were washed five times with 1% acetic acid before air drying. Bound SRB was solubilized with 200 μL of 10 mM unbuffered Tris-base solution. Absorbance was recorded, on an enzyme linked immunosorbent assay (ELISA) reader (Thermo Labsystems Multiskan EX 200–240 V), at the wavelength of 570 nm. CDDP (cis-diamminedichloroplatinum (II)) (cisplatin) was used as referent compound. Extract potency against cancer cell growth was expressed in terms of IC_{50} values (concentrations of the compound causing 50% cell growth inhibition), which were determined from the cell survival diagrams.

2.9.3. Morphological examination by light microscopy

HeLa cells (5×10^4 c/w) and MRC-5 cells (1×10^5 c/w), were seeded into 6-well plates (Thermo Scientific Nunc™), in 2 mL of the nutrient medium, and after 24 h of growth cells were exposed to 50 μg/mL of MEs of Tansy (leaves and flowers). CDDP at 1.73 μg/mL concentration was used as a referent compound. Untreated HeLa and MRC-5 cells were used as a control. Following 72 h of treatment, cells were observed under the light microscope and photographs were taken with Olympus digital camera connected to the inverted microscope (Carl Zeiss, Jena, Germany, objective 6.3/0.20).

2.10. Statistical analysis

The data were analyzed using SAS software (SAS Institute, 2002. SAS/STAT, ver. 9.00. SAS Institute Inc., Cary, NC, USA). The results represent mean values ± SE calculated from three replicate measurements. Data were subjected to square root transformation prior to analysis, followed by inverse transformation for presentation. The data were subjected to standard analysis of variance (ANOVA), and the means were separated using Fisher's LSD test ($P \leq 0.05$). Pearson Correlation Coefficients (R) and Coefficient of Determination (R^2) was used for understand an association between total phenolic content and antioxidative properties of Tansy MEs.

3. Results

3.1. Chemical composition of EO and MEs

3.1.1. Determination and quantification of EO compounds

Tansy EO was obtained after hydro-distillation from the aerial parts with the total yield of 0.32% of mass of dry plants. GC/MS analyses of EO detected 65 volatile compounds, which are listed in Table 1. Fifty eight compounds were identified and assigned to six different classes: oxygenated monoterpenes (93.5%), monoterpene hydrocarbons (3.52%), oxygenated sesquiterpenes (0.65%), sesquiterpene hydrocarbons (0.03%), aromatic hydrocarbons (1.76%), and aromatic alcohols (0.12%). The EO was characterized by a high content of the *trans*-chrysanthenyl acetate (41.37%), *trans*-chrysanthenol (12.51%), *trans*-thujone (9.04%), and *cis*-thujone (5.28%) all belonging to the oxygenated monoterpene compounds. According to the classification based on the chemical composition of EO, Tansy plants belong to the *trans*-chrysanthenyl acetate chemotype.

3.1.2. Determination of MEs compounds

The phenolic acids, flavonoids and their derivatives of Tansy MEs were evaluated by LC-DAD/ESI-TOF-MS. Results are presented in Fig. 1. Chromatograms were recorded at 280 nm. The identification of phenolic compounds was performed according to their UV–visible spectra, and the values of ion mass (m/z); names and corresponding formulas were presented in Table 2.

Phenolic acids were presented with hydroxy cinnamic acid derivatives that were detected in all analyzed MEs (Fig. 1). The most prominent compounds from this group were neochlorogenic (peak no. 2), 3,5-*O*-dicaffeoylquinic (peak no. 15) and caffeoylquinic acids (peak no. 21). Amounts of these compounds differ in MEs isolated from different parts of Tansy; thus, neochlorogenic acid was the most abundant in ME of roots, while the other parts of plant had 40–50% less quantity.

Additionally, seventeen flavonoids were recognized in investigated extracts. Some of the compounds were present in all MEs, but majority were present in a single ME. For example, isoquercetin (peak no. 7) and luteolin (peak no. 28) were detected only in ME of flower, while luteolin-7-glucoside (peak no. 12) was present in all extracts except the ME of roots. The group of eight compounds with the longest retention times was detected only in MEs from flower and leaves, but the concentrations of the most of them were significantly higher in ME of leaves. ME of leaves contained the highest amounts, particularly in regard to nepetin (peak no. 29), quercetagetin-3,6-dimethylether (peak no. 31), hispidulin (peak no. 33) and eupalitin (peak no. 34).

3.2. Total phenolic content and antioxidant activities of MEs

3.2.1. Total phenolic content (TPC)

TPC in MEs were determinate based on the absorbance values of the extract, reacted with Folin–Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents (GAE). The results of the colorimetric analysis of total phenolics are given in Table 3.

Table 1
Composition of the essential oil of *T. vulgare*.

Peak No.	Compound	KI [e]	% m/m
1	cis-Salvene	845	0.02
2	trans-Salvene	860	0.06
3	Tricyclene	916	0.04
4	α-Thujene	922	0.10
5	α-Pinene	928	1.81
6	Camphene	942	0.46
7	Thuja-2,4(10)-diene	949	0.03
8	Sabinene	969	0.18
9	β-Pinene	971	0.17
10	Dehydro-1,8-cineole	987	0.07
11	Mesitylene	993	0.06
12	Yomogi alcohol	999	0.20
13	*n.i.*	1001	0.04
14	α-Terpinene	1010	0.41
15	p-Cymene	1013	1.70
16	1,8-Cineole	1021	3.88
17	Artemisia ketone	1026	2.94
18	cis-Sabinene hydrate	1065	0.17
19	Artemisia alcohol	1083	0.12
20	Terpinolene	1084	0.25
21	trans-Sabinene hydrate	1097	0.13
22	cis-Thujone	1101	5.28
23	trans-Chrysanthenol	1112	12.51
24	trans-Thujone	1114	9.04
25	cis-p-Menth-2-en-1-ol	1119	0.35
26	Chrysanthenone	1121	0.40
27	cis-Chrysanthenol	1132	2.54
28	trans-Pinocarveol	1136	0.76
29	Camphor	1139	4.90
30	trans-Verbenol	1444	0.08
31	Pinocarvone	1158	0.68
32	Borneol	1164	2.10
33	Terpinen-4-ol	1175	2.92
34	Thuj-3-en-10-al	1183	0.03
35	p-Cymen-8-ol	1190	0.12
36	α-Terpineol	1191	0.19
37	cis-Piperitol	1195	0.13
38	Myrtenol	1197	0.04
39	Verbenone	1208	0.08
40	trans-Pulegol	1209	0.19
41	trans-Carveol	1215	0.04
42	trans-Chrysanthenyl acetate	1236	41.37
43	cis-Chrysanthenyl acetate	1258	0.25
44	n.i.	1262	0.02
45	Bornyl acetate	1282	0.74
46	trans-Linalool oxide acetate (pyranoid)	1284	0.74
47	trans-Sabinyl acetate	1291	0.03
48	n.i.	1294	0.03
49	Terpinen-4-ol acetate	1297	0.11
50	n.i.	1306	0.04
51	cis-Chrysanthenyl propionate	1322	0.28
52	α-Terpinyl acetate	1347	0.05
53	cis-Carvyl acetate	1363	0.13
54	n.i.	1367	0.18
55	Isobornyl propanoate	1372	0.02
56	n.i.	1458	0.06
57	Germacrene D	1476	0.03
58	Spathulenol	1575	0.11
59	Caryophyllene oxide	1578	0.15
60	Salvial-4(14)-en-1-one	1590	0.11
61	trans-β-Elemenone	1595	0.07
62	Ledol	1605	0.04
63	Eremoligenol	1626	0.09
64	n.i.	1647	0.05
65	neo-Intermedeol	1655	0.08
Monoterpene hydrocarbons			**3.52**
Oxygenated monoterpenes			**93.5**
Sesquiterpene hydrocarbons			**0.03**
Oxygenated sesquiterpenes			**0.65**
Aromatic hydrocarbons			**1.76**
Aromatic alcohols			**0.12**

n.i. — not identified.
KI[e] — Kovats (retention) index experimentally determined.
The chemical groups of identified compounds with corresponding portion in analyzed essential oil are presented in bold.

The TPC in MEs of Tansy stalks, flowers, leaves, and roots varied significantly from 83.6 to 221.7 mg GAE/g DW. TPC values decreased in the order: ME of roots < ME of leaves < ME of flowers < ME of stalks.

3.2.2. Radical scavenging activity

DPPH scavenging activity of tested extracts expressed as IC_{50} values varied between 44 and 80.3 μg/mL (Table 3). ME of roots showed strong potential for DPPH radical scavenging. Radical scavenging activity of other MEs was significantly lower.

Pearson Correlation Coefficient between TPC and IC_{50} values indicated strong negative correlation ($R = -0.8$) with coefficient of determination of $R^2 = 0.64$.

3.2.3. Reducing power ability

Results obtained showed that Tansy MEs have potential to act as reducing agent, increased in a concentration dependent manner (Table 3). The highest reducing power was recorded for ME of roots in all applied concentrations followed by MEs of flowers, leaves and stalks. The highest reducing power observed for ME of roots at a concentration of 1 mg/mL was almost the same as that of Trolox, with no statistically significant differences.

A strong positive correlation ($R = 0.89$; $R^2 = 0.79$) was seen between the TPC and the reducing power assay of tested extracts.

3.3. Antimicrobial activity of EO and MEs

The MIC and MBC/MFC of Tansy EO and MEs were determined against various bacteria and fungi strains. These MIC and MBC/MFC values are shown in Table 4 in comparison with values related to two antibiotics/fungicides used as references.

3.3.1. Antibacterial activity

According to the results of antibacterial activity, obtained in seven bacteria strains Tansy EO exhibited strong inhibitory effect on growth (proliferation) of Gram-negative *E. coli* and *E. cloacae*. MIC value of Tansy EO against *E. coli* and both values MIC and MBC against *E. cloacae,* were lower comparing to reference agents, streptomycin and ampicillin.

On the other hand, the comparative evaluation of antibacterial activity of MEs from Tansy stalks, leaves, flowers and roots showed a significant level of variations in different bacteria strains. MEs from stalks, leaves, flowers and roots were more effective on Gram-positive *S. aureus*, *B. cereus* and *M. flavus,* comparing to antibiotics used as references. In the present study, the Gram-negative *L. monocytogenes* was found to be the most resistant strain to the examined MEs.

3.3.2. Antifungal activity

Results of antifungal activity of tested extracts in comparison to commercial agents bifonazole and ketoconazole, are presented in Table 4. In almost all fungi tested (except *A. niger* and *T. viride*), EO showed MIC and MFC values in level comparable to bifonazole and ketoconazole. The most prominent activity of EO in microdilution test was noticed against *P. funiculosum* where MIC and MFC values were significantly lower comparing to references compounds.

Methanol extracts of *T. vulgare* showed fungistatic and fungicidal effect which varied among different fungi species. *P. funiculosum* was the most sensitive species to all MEs tested.

3.4. Antiproliferative activity of EO and MEs

3.4.1. SRB cell antiproliferative assay

Study was performed in human cervical adenocarcinoma cells (HeLa) and human fetal lung fibroblast cells (MRC-5), used as non-cancerous model for *in vitro* toxicity evaluation.

All tested MEs and EO inhibited the growth of both cancerous and non-cancerous cell lines in a dose dependent manner (data not

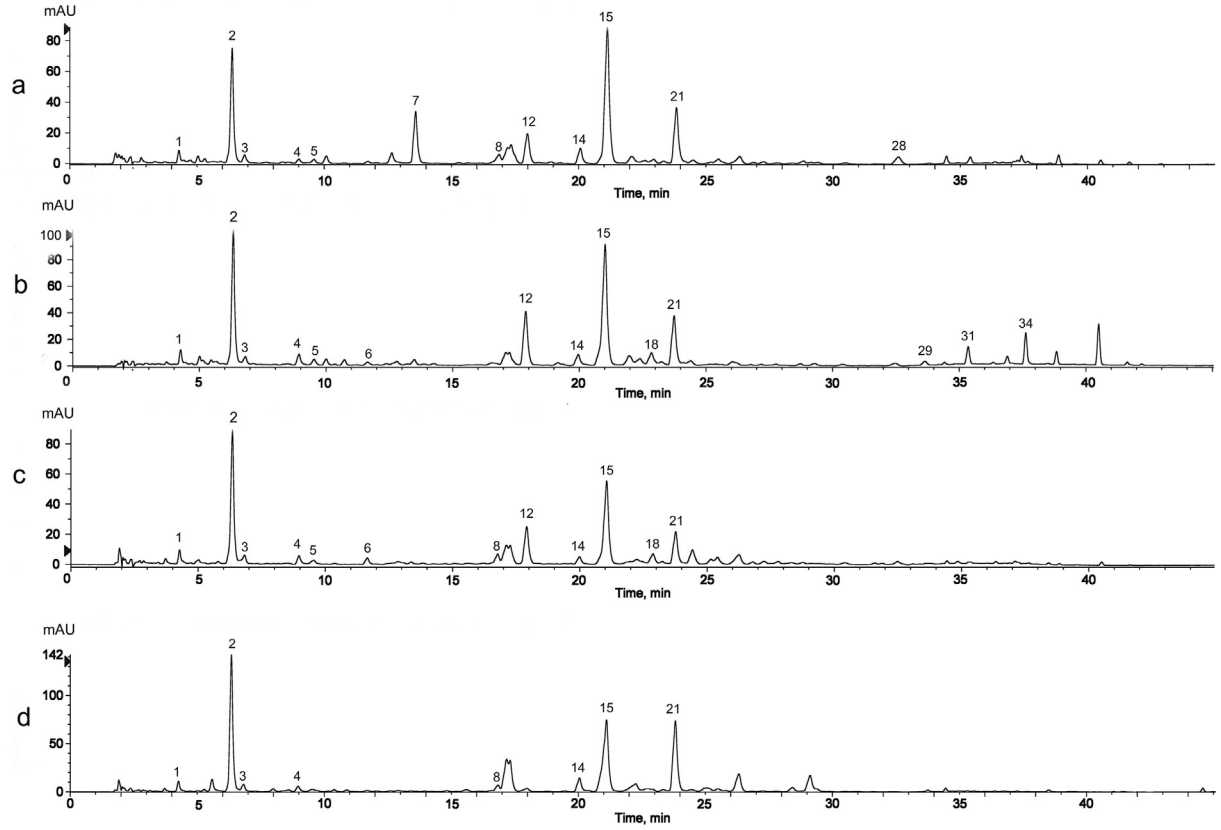

Fig. 1. Chromatograms of *T. vulgare* methanol extracts of flowers (a), leaves (b), stalks (c) and roots (d) obtained by LC-DAD/ESI-TOF-MS. Peak numbers refer to Table 2.

Table 2
Identification data of phenolic compounds found in *T. vulgare* methanol extracts by using LC-DAD/ESI-TOF-MS.

No.	UV max (nm)	Measured values (m/z)	Molecular formula	Compound name
1	240; 292sh; 326	353.0822; 354.0907; 389.0666	C16H18O9	1-Caffeoylquinic acid
2	244, sh 292,326	353.0875, 354.0902, 389.0635, 390.0672, 399.0925, 400.0960, 707.1815, 743.1561	C16H18O9	Neochlorogenic acid
3	244,sh296,326	353.0870; 354.0906; 399.0936; 707.1808	C16H18O9	Chlorogenic acid
4	244; sh294; 318	353.0872, 354.0907, 355.0928, 389.0619, 707.1832, 708.1866	C16H18O9	Cryptochlorogenic acid
5	272,322	593.1503, 594.1543, 629.1218	C27H30O15	Saponarin
6	242, sh290, 326	367.1027, 368.1068, 403.0795, 413.1090, 735.2145	C17H20O9	Caffeoyl methyl quinic acid
7	250; 344	463.0870, 464.0907, 499.0642, 500.0684, 509.0931, 510.0968	C21H20O12	Isoquercetin
8	254, 370	477.0668, 478.0704, 513.0459, 523.0718	C21H18O13	Miquelianin
9	256, sh272, 372	477.0666, 478.0702, 513.0430, 523.0727	C21H20O13	Gossypetin-8-O-glucoside
10	226, 284, 340	463.0867, 464.0901, 465.0930	C21H20O12	Quercetin-O-glycoside
11	228, 284; 336	463.0868, 464.0904, 465.0930	C21H20O12	Quercetin-O-glucoside
12	254; sh268; 350	447.0910; 448.0947; 483.0666; 493.0970; 895.1887	C21H20O11	Luteolin-7-glucoside
13	254,sh272,348	461.0701, 462.0735, 497.0467, 507.0757, 923.1461	C21H18O12	Luteolin-7-O-glucuronide
14	244,sh292,326	353.1186, 516.1226, 1031.2471, 1032.2511	C25H24O12	3,4-O-dicaffeoylquinic acid
15	242,sh292,326	515.1187, 516.1217, 517.1243	C25H24O12	3,5-O-dicaffeoylquinic acid
16	242,sh292,322	515.1180, 516.1222, 551.0952, 552.0994	C25H24O12	4,5-O-dicaffeoylquinic acid
17	264, 326	461.0718, 462.0752, 497.0480, 507.0780	C21H18O12	Scutellarin
18	256, sh268,354	447.1029, 478.1065, 513.0818	C22H22O12	Petunidin-3-O-glucoside
19	266,336	445.0767, 446.0806, 481.0534, 891.1628	C21H18O11	Baicalin
20	242, sh290,326	469.1185, 515.1193, 516.1219, 517.1244	C24H22O10	nd
21	244, sh294,328	515.1184, 516.1217, 1031.2444, 1032.2483	C25H24O12	Dicaffeoylquinic acid
22	254; sh 268; 354	475.0872; 476.0908; 521.0951; 951.1824	C22H20O12	nd
23	266,344	475.0875, 476.0912, 511.0642, 521.0939	C22H24O12	nd
24	236, 294, 324	461.1082; 462.1123; 497.0856	C22H22O11	nd
25	228, 284	477.1025, 478.1062, 513.0797, 955.2156	C22H22O13	nd
26	254, sh272,356	505.0975, 506.1011, 541.0762	C23H22O13	nd
27	256, sh279,356	505.0974, 506.1011, 541.0750	C23H22O13	nd
28	254. 264, 348	285.0398; 286.0435; 321.0184; 331.0477; 571.0880	C15H10O6	Luteolin
29	256.272.346	315.0505; 316.538; 351.0262	C16H12O7	Nepetin
30	242, sh292, 328	667.1503, 678.1539, 1355.3087, 1356.3113	C34H30O15	3,4,5-tricaffeoylquinic acid
31	256, sh274,352	345.0606, 346.0640, 381.0378, 391.0667	C17H14O8	Quercetagetin-3,6-dimethylether
32	266, 336	269.0453; 270.0482; 271.0511	C15H10O5	Apigenin
33	274, 336	299.0554, 300.0590, 335.0331, 345.0606	C16H12O6	Hispidulin
34	274, 344	329.0661, 330.0695, 365.0433, 375.0713	C17H14O7	Eupalitin
35	256, 350	359.0762, 360.0801, 405.0865	C18H16O8	nd
36	274, 342	297.0795, 343.0814, 344.0847	C17H14O5	nd

Nd — not detected.

Table 3
The total phenolic content (TPC), DPPH assay (expressed as IC_{50} value) and reducing power ability (RPA) of the methanol extracts (MEs) from the different parts of *T. vulgare*.

ME	TPC (mgGAE/g)	IC_{50} (μg/mL)	RPA (A_{700})				
			0.1 mg/mL	0.25 mg/mL	0.5 mg/mL	0.75 mg/mL	1 mg/mL
Roots	221.7^{a}	44^{c}	0.123^{b}	0.363^{b}	0.602^{b}	0.796^{b}	0.903^{a}
Leaves	112.6^{b}	77^{a}	0.029^{d}	0.269^{c}	0.451^{c}	0.62^{c}	0.681^{b}
Flowers	96.2^{c}	58.3^{b}	0.114^{bc}	0.243^{d}	0.431^{cd}	0.494^{e}	0.598^{c}
Stalks	83.6^{d}	80.3^{a}	0.109^{c}	0.237^{d}	0.407^{d}	0.551^{d}	0.649^{d}
Trolox	/	17.46^{d}	0.346^{a}	0.549^{a}	0.824^{a}	0.92^{a}	0.926^{a}

Within each column, values with the same letter are not significantly different at the $p \leq 0.05$ level according to the LSD test.

shown). The IC_{50} values, for all MEs and EO, are summarized in Fig. 2e and j. MEs of leaves and flowers exhibited antiproliferative activity in micrograms range (up to 100 μg/mL), in both HeLa and MRC-5 cells. Antiproliferative activity of other two MEs and EO in both cell lines was significantly lower. EO and ME of root significantly inhibited proliferation of HeLa cells, almost twice as stronger compared to MRC-5 cells, which suggested certain tumor-cell selective potential.

3.4.2. Morphological examination of cells

Effect of MEs of leaves and flowers on morphological changes of human tumor cells (HeLa), and in human non-tumor cells (MRC-5), was observed under inverted microscope. MEs of leaves and flowers were selected for morphological study *in vitro*, since they showed significant antproliferative effect (SRB test). As shown in Fig. 2a, HeLa cells in the control group showed regular polygonal morphology, with normal cell surface extensions (lamellipodia), and only a few cells were round. Following the treatment by MEs of leaves and flowers (50 μg/mL), HeLa cells were found mainly floating, as the result of cell death. In addition, cells that still stayed attached appeared shrunken and rounded (Fig. 2c, d). This effect was comparable to cisplatin action (Fig. 2b) at concentration corresponding to its IC_{50} value in HeLa cells (1.7 μg/mL) and was characteristic for apoptotic cell death. Interestingly, MEs of leaves and flowers, didn't lead to a significant cell detachment of MRC-5 cells (Fig. 2h, i), under the same treatment conditions. Although, cell started to elongate, change their shape and obviously proliferated less.

Morphological changes of MRC-5 cells, such as cell shrinkage and detachment, were however notable following the treatment with CDDP (Fig. 2g).

4. Discussion

Commercially available Tansy EOs, are mostly of the thujone type. The U.S. Food and Drug Administration limited the use of Tansy in alcoholic beverages due to documented toxic effects of thujone (FDA, 2015). European Medicinal Agency and European Commission allow the presence of thujone in beverages in most European countries but recommended amount of thujone in a preparation needs to be specified and that exposures in the range between 3 and 7 mg/day do not pose special concerns (EMA, 2011). The amount of dietary intake of 1 mg on average may not cause special concerns (EMA, 2011).

In order to identify the most effective but concurrently safe extract for human consumption, we screened and compared chemical composition and biological properties of EO and MEs of Tansy plants.

The yield and composition of Tansy oils are known for highly intraspecific variability due to genetical variation and/or environmental conditions, and different chemotypes are determinate according to the most dominant constituent. The EO isolated from the aerial parts of Tansy plants collected near Belgrade, Serbia yield was 0.32%. The EO yield from wild populations of Tansy from 40 different locations in Norway was in the range of 0.35–1.90% (*v*/*w*) (Rohloff et al., 2004). Another study reported that EO yields of 5 different Tansy genotypes during all vegetative period were 0.14–0.45% and 0.35–0.79% in dried leaves and flowers, respectively (Dragland et al., 2005).

According to the revealed chemical composition, Tansy EO was rich in oxygenated monoterpenes and belongs to the *trans*-chrysanthenyl acetate chemotype, with almost 42% of this constituent. Our findings are in agreement with data provided by Popov et al. (2001) that investigated chemical composition of Tansy oil from eight different localities

Table 4
Antimicrobial activity of essential oil (EO) and methanol extracts (MEs) from the different parts of *T. vulgare*.

Bacteria	MIC/MBC (mg/mL)													
	EO		ME stalks		ME leaves		ME flowers		ME roots		Streptomycine		Ampiciline	
Staphylococcus aureus	0.21^{d}	0.85^{d}	0.09^{c}	0.18^{b}	0.05^{b}	0.09^{a}	0.05^{b}	0.09^{a}	0.09^{c}	0.18^{b}	0.04^{a}	0.09^{a}	0.25^{d}	0.37^{c}
Bacillus cereus	0.21^{d}	0.85^{d}	0.09^{c}	0.18^{b}	0.05^{b}	0.09^{a}	0.02^{a}	0.09^{a}	0.09^{c}	0.18^{b}	0.09^{c}	0.17^{b}	0.25^{d}	0.37^{c}
Micrococcus flavus	0.64^{f}	0.85^{d}	0.09^{c}	0.18^{b}	0.05^{b}	0.09^{a}	0.02^{a}	0.09^{a}	0.09^{c}	0.18^{b}	0.17^{cd}	0.34^{c}	0.25^{e}	0.37^{c}
Listeria monocytogenes	2.12^{e}	16.93^{d}	0.38^{c}	0.75^{b}	0.38^{c}	0.75^{b}	0.02^{a}	0.75^{b}	0.75^{d}	1.5^{c}	0.17^{b}	0.34^{a}	0.37^{c}	0.49^{ab}
Pseudomonas aeruginosa	8.47^{e}	16.93^{f}	0.38^{c}	0.75^{c}	0.38^{c}	0.94^{d}	0.18^{b}	0.38^{b}	0.09^{a}	0.18^{a}	0.17^{b}	0.34^{b}	0.74^{d}	1.24^{e}
Salmonella typhimurium	2.12^{c}	8.47^{c}	0.38^{b}	0.75^{b}	0.18^{a}	0.38^{a}	0.18^{a}	0.38^{b}	0.38^{b}	0.75^{b}	0.17^{a}	0.34^{a}	0.37^{b}	0.49^{ab}
Escherichia coli	0.03^{a}	0.53^{d}	0.18^{c}	0.38^{b}	0.18^{c}	0.38^{b}	0.09^{b}	0.18^{a}	0.18^{c}	0.38^{b}	0.17^{c}	0.34^{b}	0.25^{d}	0.49^{c}
Enterobacter cloacae	0.11^{ab}	0.42^{bc}	0.38^{d}	0.75^{d}	0.18^{b}	0.38^{b}	0.09^{a}	0.18^{a}	0.18^{b}	0.38^{b}	0.26^{c}	0.52^{c}	0.37^{d}	0.74^{d}
Fungi	MIC/MFC (mg/mL)										Bifonazole		Ketokonazole	
Aspergillus fumigatus	0.13^{a}	0.51^{c}	0.13^{a}	0.45^{b}	0.13^{a}	0.23^{a}	0.23^{b}	0.45^{b}	0.13^{a}	0.23^{a}	0.15^{ab}	0.2^{a}	0.2^{b}	0.5^{c}
Aspergillus versicolor	0.11^{bc}	0.21^{b}	0.03^{a}	0.06^{a}	0.15^{c}	0.3^{c}	0.08^{b}	0.3^{c}	0.15^{c}	0.6^{d}	0.1^{bc}	0.2^{b}	0.2^{d}	0.5^{cd}
Aspergillus ochraceus	0.05^{a}	0.21^{a}	0.3^{c}	0.6^{c}	0.15^{b}	0.6^{c}	0.15^{b}	0.3^{b}	0.15^{b}	0.6^{c}	0.15^{b}	0.2^{a}	1.5^{d}	2^{d}
Aspergillus niger	0.25^{d}	0.51^{c}	1^{e}	2^{d}	0.13^{b}	0.23^{b}	0.13^{b}	0.23^{b}	0.02^{a}	0.03^{a}	0.15^{b}	0.2^{b}	0.2^{c}	0.5^{c}
Trichoderma viride	0.42^{d}	8.47^{d}	0.03^{a}	0.23^{a}	0.3^{c}	0.6^{b}	0.15^{b}	0.6^{b}	0.3^{c}	0.6^{b}	0.15^{b}	0.2^{a}	1^{e}	1^{c}
Penicillium funiculosum	0.002^{a}	0.003^{a}	0.04^{b}	0.15^{b}	0.04^{b}	0.15^{b}	0.03^{ab}	0.45^{e}	0.04^{b}	0.3^{d}	0.2c	0.25^{c}	0.2^{c}	0.5^{f}
Penicillium ochrochloron	0.11^{a}	0.22^{a}	0.3^{c}	0.6^{c}	0.3^{c}	0.6^{c}	0.15^{a}	0.3^{b}	0.3^{c}	0.6^{c}	0.2^{b}	0.25^{ab}	2.5^{d}	3.5^{d}
Penicillium verrucosum	0.11^{b}	022^{bc}	023^{c}	0.45^{d}	0.13^{b}	0.23^{bc}	0.13^{b}	0.23^{bc}	0.02^{a}	0.03^{a}	0.10^{b}	0.20^{b}	0.20^{c}	0.30^{c}

Values with the same letter for MIC/ MBC/ MFC data within each row are not significantly different at the $p \leq 0.05$ level according to the LSD test.

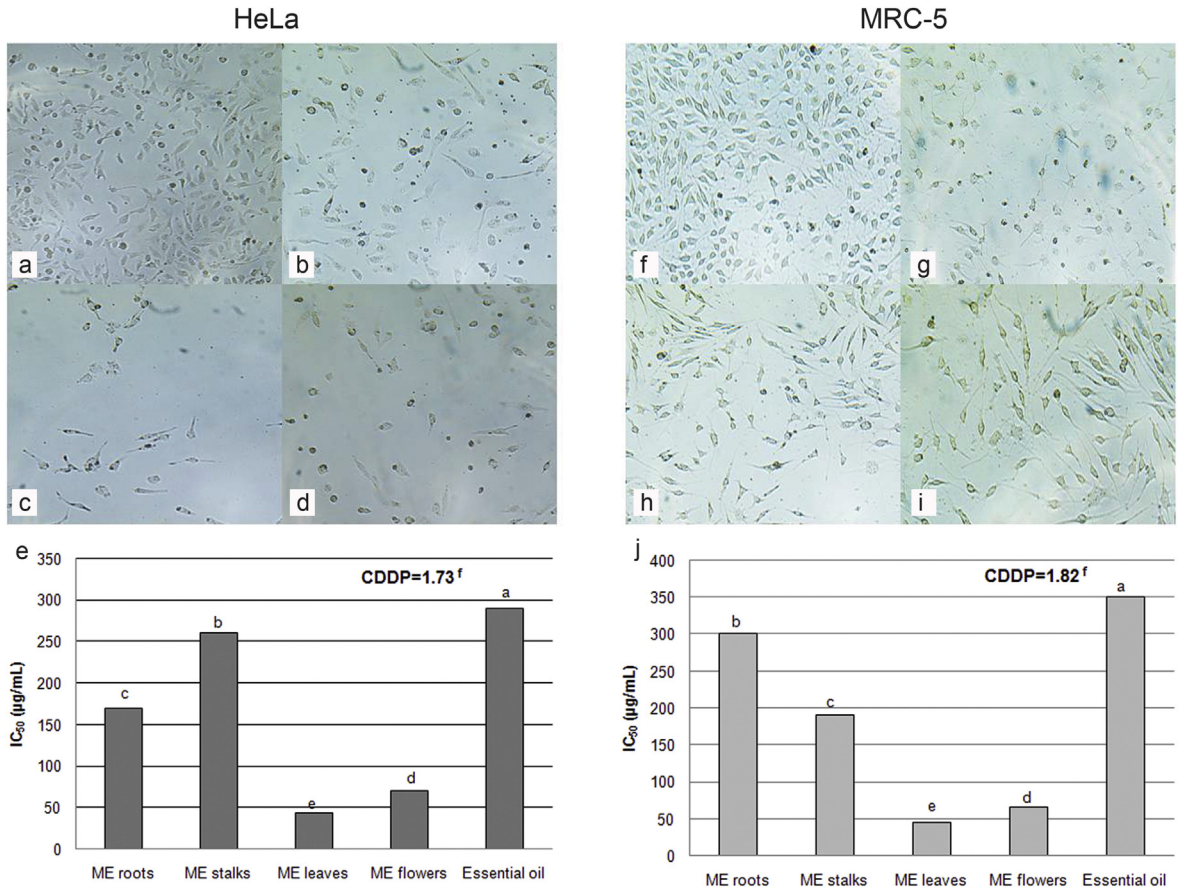

Fig. 2. Comparative presentation of photomicrographs and IC_{50} values (μg/mL), obtained, for 72 h of treatment in HeLa and MRC-5 cells. Photomicrographs present morphology of untreated HeLa cells (a), HeLa cells treated with: 1.7 μg CDDP (b), ME of leaves (c), ME of flowers (d). Morphology of untreated MRC-5 cells (f), MRC-5 cells treated with: 1.7 μg CDDP (g), ME of leaves (h), ME of flowers (i). Graphs present corresponding antiproliferative activities in terms of IC_{50} values (μg/mL), obtained in HeLa (e), and MRC-5 cells (j).

in Serbia and revealed the presence of thujone, *trans*-chrysanthenyl acetate, chrysanthenyl and camphor chemotype.

In order to investigate the biological properties of EO, we exposed some of the food-borne pathogens that menace to the food safety. It is known that majority of terpenes and terpenoids from EOs could be active against bacteria (Mikulašová and Vaverková, 2009; Móricz et al., 2015). Therefore, we can assume that *trans*- chrysanthenyl acetate as dominant oil compound, greatly contributes to antimicrobial activity of the tested oil. In the present study Tansy EO exhibited antifungal effect against the panel of eight fungi. The most prominent activity of EO was noticed against *P. funiculosum* where MIC and MFC values were significantly lower than those of commercial agents bifonazole and ketokonazole. Moreover, EO showed undoubted biological activity against Gram-negative *E. coli* and *E. cloacae*. Several mechanisms are proposed in the literature to explain the action of EO constituents in microbial cells (Burt, 2004). EOs can degrade the cell wall due to hydrophobic interaction with phospholipid bilayer of cytoplasmic membrane, leading to increased permeability and a loss of cellular constituents. Also, they can disrupt electron flow and active transport or affect variety of enzyme systems. Our results are in accordance with previous reports showing that EOs may exhibit greater activity against Gram-positive than Gram-negative bacteria, which possess an outer lipopolysaccharide covering, that restricts diffusion of hydrophobic compounds (Shelef et al., 1984; Ouattara et al., 1997; Smith-Palmer et al., 1998; Lambert et al., 2001; Pintore et al., 2002; Harpaz et al., 2003).

However, certain small terpenoid and phenolic compounds have been reported to disintegrate the outer membrane of Gram–negative bacteria and affect the growth of microbial cells (Burt, 2004). Moreover, methanol extracts from different parts of Tansy plant have been shown to be rich in phenolic acids, which act as highly effective free radical scavengers. In all tested MEs, hydroxy cinnamic acid derivatives (neochlorogenic, 3,5-*O*-dicaffeoylquinic and caffeoylquinic acids) were the major compounds. Like all phenolic acids that possess an *ortho*- dihydroxy group acting as a putative radical target site, the major antiradical activities of MEs is proposed to be related to these compounds (Foti et al., 1996; Cao et al., 1997).

A statistically significant correlation between the TPC values and antioxidant properties of tested extracts was observed in the present study. ME of roots exhibited the strongest DPPH radical scavenging activity, which is obviously related to the highest content of phenols, neochlorogens, 3,5-O-dicaffeoylquinic and dicaffeoylquinic acids, detected by LC-DAD/ESI-TOF-MS study. ME of flowers also demonstrated certain DPPH radical scavenging activity, which may be attributed to the presence of isoquercetin, spotted only in this extract. Still, the obtained TPC values of *T. vulgare* extracts tested were higher, while DPPH-IC_{50} values were lower, than those reported in literature for *T. vulgare* and other *Tanacetum* species. (Kähkoänen et al., 1999; Wu et al., 2006; Juan-Badaturuge et al., 2009; Esmaeili et al., 2010; Baranauskienė et al., 2014).

According to previous reports, mechanisms responsible for phenolic biological activity may include enzyme inhibition (Mason and Wasserman, 1987). Comparative analysis of antimicrobial action of Tansy MEs extracts in the present study, showed stronger antimicrobial effect in Gram-positive bacteria, comparing to streptomycine and ampiciline. The strongest antimicrobial activity was observed in ME of flowers (against *E. coli* and *E. cloacae*). In addition, MEs of Tansy

demonstrated both fungistatic and fungicidal effect. ME of flowers particularly exhibited lower values of MIC and MFC than ketoconazole against all tested fungi species.

Investigation of Tansy antiproliferative effects in human cells has been limited to a few studies so far. Certain *in vitro* cytotoxicity of Tansy ethanol extracts was reported in tumor cell lines derived from colon, breast, lung and human acute T leukemia cell line (Ramírez-Erosa et al., 2007; Wegiera et al., 2012; Gospodinova et al., 2014). However, there are variations in reported IC_{50}, which can be explained by intragenus and intra-species diversity in chemical composition of plants from different geographical areas. In addition, antiproliferative effect was tested in normal human embryonic lung fibroblast cells (MRC-5). Obtained results revealed that MEs of leaves and flowers exhibited the strongest inhibitory effect on growth and proliferation of human cells *in vitro*, with IC_{50} values up to 100 μg/mL. The leaves extract had the strongest antiprolifetrative effect in tumor HeLa cells, which is possibly due to nepetin, hispidulin and eupalitin, detected only in this extract. Previous studies showed that these compounds had cytotoxic properties (Militão et al., 2004; Kuroda et al., 2007; Ghalib et al., 2013). Interestingly, Tansy EO demonstrated tumor-cell selective potential, being less cytotoxic in non-tumor MRC-5 cells, than in HeLa.

5. Conclusion

In conclusions, our findings suggest that *Tanacetum vulgare* presents a good source of valuable biologically active substances with antibacterial and antifungal properties. This suggests methanol extracts and essential oil of *Tanacetum vulgare* as natural additives in food preservation process. To the best of our knowledge this is the first report of *in-vitro* antiproliferative effect of Tansy extracts and EO, in human tumor cervix carcinoma cells (HeLa). Additionally, the Tansy MEs possess high antioxidant potential with phenolic acids being a major radical scavenging contributor with the proved antiproliferative activity to HeLa cells. Investigated extracts and essential oil with the low thujone content in the current study can provide a very promising and effective alternative in the field of antimicrobial applications. However, observed antimicrobial effects of Tansy EO and MEs, require further validation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.sajb.2017.03.028.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. fourth ed. Allured Publishing Corporation, Carol Stream, Illinois, USA.
Baranauskienė, R., Kazernavičiūtė, R., Pukalskienė, M., Maždžierienė, R., Venskutonis, P.R., 2014. Agrorefinery of *Tanacetum vulgare* L. into valuable products and evaluation of their antioxidant properties and phytochemical composition. Industrial Crops and Products 60, 113–122.
Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use a free radical method to evaluate antioxidative activity. LWT- Food Science and Technology 28, 25–30.
Brown, A.M.G., Edwards, C.M., Davey, M.R., Power, J.B., Lowe, K.C., 1997. Effects of extracts of *Tanacetum* species on human polymorphonuclear leucocyte activity *in vitro*. Phytotherapy Research 11, 479–484.
Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods — a review. International Journal of Food Microbiology 94, 223–253.
Cao, G., Sofic, E., Prior, R.L., 1997. Antioxidant and prooxidant behavior of flavonoids: structure–activity relationships. Free Radical Biology and Medicine 22, 749–760.
Chiasson, H., Belanger, A., Bostanian, N., Vincent, C., Poliquin, A., 2001. Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* (Asteraceae) essential oils obtained by three methods of extraction. Journal of Economic Entomology 94, 167–171.
CLSI, 2009. Clinical and laboratory standards institute methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. CLSI Publication M07–A8, eighth ed. Clinical and Laboratory Standards Institute, Wayne, PA.
Collin, G.J., Deslauriers, H., Pageau, N., Gagnon, M., 1993. Essential oil of Tansy (*Tanacetum vulgare* L.) of Canadian origin. Journal of Essential Oil Research 5, 629–638.
Dragland, S., Rohloff, J., Mordal, R., Iversen, T.H., 2005. Harvest regimen optimization and essential oil production in five tansy (*Tanacetum vulgare* L.) genotypes under a northern climate. Journal of Agriculture and Food Chemistry 53, 4946–4953.
EMA (European Medicines Agency), 2011. Public Statement on the Use of Herbal Medicinal Products Containing Thujone (Doc. Ref. EMA/HMPC/732886/2010).
Esmaeili, A.M., Sonboli, A., Noushabadi, M.A., 2010. Antioxidant and protective properties of six Tanacetum species against hydrogen peroxide-induced oxidative stress in K562 cell line: a comparative study. Food Chemistry 121, 148–155.
Espinel-Ingroff, A., 2001. Comparison of the E-test with the NCCLS M38-P method for antifungal susceptibility testing of common and emerging pathogenic filamentous fungi. Journal of Clinical Microbiology 39, 1360–1367.
Evans, W.C., 1996. Trease and Evans' Pharmacognosy. 14th ed. WB Saunders, London, p. 288.
Foti, M., Piattelli, M., Baratta, M.T., Ruberto, G., 1996. Flavonoids, coumarins, and cinnamic acids as antioxidants in a micellar system. Structure–activity relationship. Journal of Agricultural and Food Chemistry 44, 497–501.
Gallino, M., 1988. Essential oil from *Tanacetum vulgare* growing spontaneously in 'Tierra del Fuego' (Argentina). Planta Medica 54, 182.
Ghalib, R.M., Mehdi, S.H., Hashim, R., Sulaiman, O., Foong, F.H.N., Ahamed, B.M.K., Majid, A.M.S.A., Ahmed, F., 2013. Eupalitin from *Asparagus falcatus* (Linn.) has anticancer activity and induces activation of caspases 3/7 in human colorectal tumor cells. Journal of Medicinal Plant Research 7, 1401–1405.
Gospodinova, Z., Antov, G., Angelova, S., Krasteva, M., 2014. *In vitro* antitumor potential of Bulgarian *Tanacetum vulgare* L. on human breast adenocarcinoma cells. International Journal of Pharma Science 4, 468–472.
Grieve, M., 1984. Tansy. In: Leyel, C.F. (Ed.), A Modern Herbal. Penguin Books Ltd, Middlesex, pp. 789–790.
Harpaz, S., Glatman, L., Drabkin, V., Gelman, A., 2003. Effects of herbal essential oils used to extend the shelf life of fresh water reared Asian sea bass fish (*Lates calcarifer*). Journal of Food Protection 66, 410–417.
Haughton, C.S., 1978. Green Immigrants: The Plants that Transformed America. Harcourt Brace Jovanovich, Inc., New York.
Heywood, V.H., 1976. Tanacetum L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea, Plantaginaceae to Compositae (and Rubiaceae) vol. 4. Cambridge University Press, Cambridge, pp. 169–171.
Holetz, F.B., Pessini, G.L., Sanches, N.R., Cortez Diogenes, A.G., Nakamura, C.V., 2002. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Memórias do Instituto Oswaldo Cruz 97, 1027–1031.
Holopainen, M., 1989. A Study on the Essential Oil of Tansy (*Tanacetum vulgare* L.). (Ph.D. Thesis). Department of Pharmacy, University of Helsinki, Finland.
Holopainen, M., Kauppinen, V., 1989. Antimicrobial activity of different chemotypes of Finnish Tansy. Planta Medica 55, 103.
Juan-Badaturuge, M., Habtemariam, S., Jackson, C., Thomas, M.J.K., 2009. Antioxidant principles of *Tanacetum vulgare* L. aerial parts. Natural Product Communications 4, 1561–1564.
Kähkoänen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry 47, 3954–3962.
Kuroda, M., Yokosuka, A., Kobayashi, R., Jitsuno, M., Kando, H., Nosaka, K., Ishii, H., Yamori, T., Mimaki, Y., 2007. Sesquiterpenoids and flavonoids from the aerial parts of *Tithonia diversifolia* and their cytotoxic activity. Chemical and Pharmaceutical Bulletin 55, 1240–1244.
Lahlou, S., Tahraoui, A., Israili, Z., Lyoussi, B., 2007. Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. Journal of Ethnopharmacology 110, 458–463.
Lambert, R.J.W., Skandamis, P.N., Coote, P., Nychas, G.J.E., 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology 91, 453–462.
Lawrence, B.M., 1992. Essential oils as source of natural aroma chemicals. Perfumer & Flavorist 17, 15–28.
Ljaljević-Grbić, M., Stupar, M., Vukojević, J., Soković, M., Mišić, D., Grubišić, D., Ristić, M., 2008. Antifungal activity of *Nepeta rtanjensis* essential oil. Journal of the Serbian Chemical Society 73, 961–965.
Mabey, R., 1996. Flora Botanica. The Definitive Guide to Wild Flowers, Plants and Trees. Sinclair-Stevenson, London, UK.
Mason, T.L., Wasserman, B.P., 1987. Inactivation of red beet beta-glucan synthase by native and oxidized phenolic compounds. Phytochemistry 26, 2197–2202.
Mikulašová, M., Vaverková, Š., 2009. Antimicrobial effects of essential oils from *Tanacetum vulgare* L. and *Salvia officinalis* L., growing in Slovakia. Nova Biotechnologica et Chimica 9, 161–166.
Militão, G.C., Albuquerque, M.R., Pessoa, O.D., Pessoa, C., Moraes, M.E., de Moraes, M.O., Costa-Lotufo, L.V., 2004. Cytotoxic activity of nepetin, a flavonoid from *Eupatorium ballotaefolium* HBK. Pharmazie 59, 965–966.
Mitich, L.W., 1992. Intriguing world of weeds — Tansy. Weed Technology 6, 242–244.
Mordujovich-Buschiazzo, P., Balsa, E.M., Busciazzo, H.O., Mandrile, E., Rosella, M., Schinella, G., Fioravanti, D., 1996. Anti-inflammatory activity of *Tanacetum vulgare*. Fitoterapia 67, 319–322.
Móricz, M.A., Häbe, T.T., Böszörményi, A., Ott, G.P., 2015. Tracking and identification of antibacterial components in the essential oil of *Tanacetum vulgare* L. by combination of high-performance thin-layer chromatography with direct bioautography and mass spectrometry. Journal of Chromatography 1422, 310–317.

Nano, G.M., Bicchi, C., Frattini, C., Gallino, M., 1979. Wild Piedmontese plants II. A rare chemotype of *Tanacetum vulgare* L., abundant in Piedmont (Italy). Planta Medica 35, 270–274.

Neszmélyi, A., Milne, G.W.A., Héthelyi, E.Â., 1992. Composition of the essential oil of clone 409 of *Tanacetum vulgare* and 2D NMR investigation of trans-chrysanthenyl acetate. Journal of Essential Oil Research 4, 243–250.

Ouattara, B., Simard, R.E., Holley, R.A., Piette, G.J.P., Bégin, A., 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. International Journal of Food Microbiology 37, 155–162.

Papazisis, K.T., Geromichalos, G.D., Dimitriadis, K.A., Kortsaris, A.H., 1997. Optimization of the sulforhodamine B colorimetric assay. Journal of Immunological Methods 208, 151–158.

Perez, R.P., Godwin, A.K., Handel, L.M., Hamilton, T.C., 1993. A comparison of clonogenic, microtetrazolium and sulforhodamine B assays for determination of cisplatin cytotoxicity in human ovarian carcinoma cell lines. European Journal of Cancer 29A, 395–399.

Pintore, G., Usai, M., Bradesi, P., Juliano, C., Boatto, G., Tomi, F., Chessa, M., Cerri, R., Casanova, J., 2002. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. Flavour and Fragrance Journal 17, 15–19.

Popov, V., Ristić, M., Gašić, O., Sekulović, D., 2001. Varijabilnost etarskog ulja *Tanacetum vulgare*. Lekovite Sirovine 20, 45–50.

Ramírez-Erosa, I., Huang, Y., Hickie, R.A., Sutherland, R.G., Barl, B., 2007. Xanthatin and xanthinosin from the burs of *Xanthium strumarium* L. as potential anticancer agents. Canadian Journal of Physiology and Pharmacology 85, 1160–1172.

Rohloff, J., Mordal, R., Dragland, S., 2004. Chemotypical variation of Tansy (*Tanacetum vulgare* L.) from 40 different locations in Norway. Journal of Agricultural and Food Chemistry 52, 1742–1748.

Schearer, W.R., 1984. Components of oil of Tansy (*Tanacetum vulgare)* that repel Colorado potato beetles (*Leptinotarsa decemlineara*). Journal of Natural Products 47, 964–969.

Shelef, L.A., Jyothi, E.K., Bulgarelli, M.A., 1984. Growth of enteropathogenic and spoilage bacteria in sage-containing broth and foods. Journal of Food Science 49, 737–740.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16, 144–153.

Sirisoma, N.S., Hold, K.M., Casida, J.E., 2001. a- and b-Thujones (herbal medicines and food additives): synthesis and analysis of hydroxy and dehydrometabolites. Journal of Agricultural and Food Chemistry 49, 1915–1922.

Smith-Palmer, A., Stewart, J., Fyfe, L., 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Letters of Food Microbiology 26, 118–122.

Tsukatani, T., Suenaga, H., Shiga, M., Noguchi, K., Ishiyama, M., Ezoe, T., Matsumoto, K., 2012. Comparison of WST-8 colorimetric method and CLSI broth microdilution method for susceptibility testing against resistant bacteria. Journal of Microbiological Methods 90, 160–166.

U.S. FDA (Food and Drug Administration), 2015. Food Additives Permitted for Direct Addition to Food for Human Consumption (Revised as of April 1, 2015).

Wegiera, M., Smolarz, H.D., Druch, M.J., Korczak, M., Koproń, K., 2012. Cytotoxic effect of some medicinal plants from Asteraceae family on J-45.01 leukemic cell line-pilot study. Acta Poloniae Pharmaceutica 69, 263–268.

Williams, C.A., Harborne, J.B., Geiger, H., Hoult, J.R., 1999. The flavonoids of *Tanacetum parthenium* and *T. vulgare* and their anti-inflammatory properties. Phytochemistry 51, 417–423.

Woolf, A., 1999. Essential oil poisoning. Journal of Toxicology. Clinical Toxicology 37, 721–727.

Wu, C., Chen, F., Wang, X., Kim, H.J., He, G., Haley-Zitlin, V., Huang, G., 2006. Antioxidant constituents in feverfew (*Tanacetum parthenium*) extract and their chromatographic quantification. Food Chemistry 96, 220–227.

Xie, G., Schepetkin, I.A., Quinn, M.T., 2007. Immunomodulatory activity of acidic polysaccharides isolated from *Tanacetum vulgare* L. International Immunopharmacology 7, 1639–1650.

Xue, L., Yongkang, L., Huixing, S., Juan, Y., 2011. Antioxidant activities and functional properties of grass carp (*Ctenopharyngodon idellus*) protein hydrolysates. Journal of Science and Food Agriculture 92, 292–298.

Zaurov, D.E., Belolipov, I.V., Kurmukov, A.G., Sodombekov, I.S., Akimaliev, A.A., Eisenman, S.W., 2013. The medicinal plants of Uzbekistan and Kyrgyzstan. In: Eisenman, S.W., Zaurov, D.E., Struwe, L. (Eds.), Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan. Springer, New York, p. 235.