



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

N. Devrnja^{a,*}, B. Anđelković^b, S. Arandžević^c, S. Radulović^c, M. Soković^a, D. Krstić-Milošević^a, M. Ristić^d, D. Čalić^a

^a University of Belgrade, Institute for Biological Research “Siniša Stanković”, Bulevar despota Stefana 142, 11060 Belgrade, Serbia

^b University of Belgrade, Faculty of Chemistry, Studentski trg 16, 11000 Belgrade, Serbia

^c Institute for Oncology and Radiology of Serbia, Pasterova 14, 11000, Belgrade, Serbia

^d University of Belgrade, Institute for Medical Plants Research “Dr Josif Pančić”, Tadeuša Koščuška 1, 11000 Belgrade, Serbia

ARTICLE INFO

Article history:

Received 5 October 2016

Received in revised form 10 January 2017

Accepted 9 March 2017

Available online 28 March 2017

Edited by J Van Staden

Keywords:

Tanacetum vulgare L.

Essential oil

Methanol extracts

Antimicrobial effect

Antioxidant activity

Cytotoxicity

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.

© 2017 SAAB. Published by Elsevier B.V. All rights reserved.

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 *Tanacetum 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-Buschiazzi 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

* Corresponding author at: University of Belgrade, Institute for Biological Research “Siniša Stanković”, Department of Plant Physiology, Bulevar despota Stefana 142, 11060 Belgrade, Serbia.

E-mail address: nina.devrnja@ibiss.bg.ac.rs (N. Devrnja).

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 (Scheerer, 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 iodinitrotetrazolium 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 (H₂) 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 iodinitrotetrazolium 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% CO₂.

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 IC₅₀ 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 (R²) 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), quercetagenin-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 IC₅₀ 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 IC₅₀ 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. funiculosus* 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. funiculosus* 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

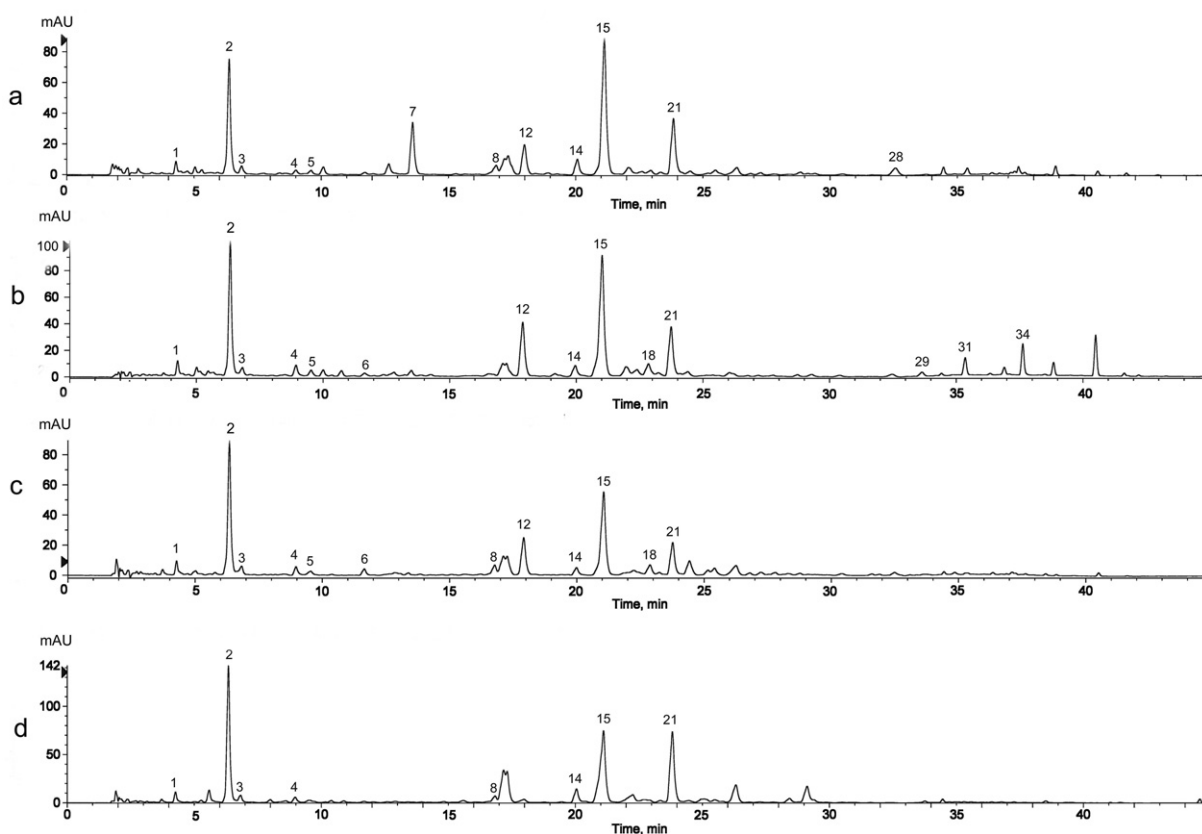


Fig. 1. Chromatograms of *T. vulgare* methanol extracts of flowers (a), leaves (b), stalks (c) and roots (d) obtained by LC-DAD/ESI-TOF-MS. Peak numbers refer to Table 2.

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-glycoside |
| 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 | Quercetagenin-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 IC₅₀ 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 IC₅₀ 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 antiproliferative 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 IC₅₀ 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 intra-specific 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 | | | | | | | |
| <i>Staphylococcus aureus</i> | 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 |
| <i>Bacillus cereus</i> | 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 |
| <i>Micrococcus flavus</i> | 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 |
| <i>Listeria monocytogenes</i> | 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} |
| <i>Pseudomonas aeruginosa</i> | 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 |
| <i>Salmonella typhimurium</i> | 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} |
| <i>Escherichia coli</i> | 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 |
| <i>Enterobacter cloacae</i> | 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) | | | | | | | | | | | | | |
| <i>Aspergillus fumigatus</i> | 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 |
| <i>Aspergillus versicolor</i> | 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} |
| <i>Aspergillus ochraceus</i> | 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 |
| <i>Aspergillus niger</i> | 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 |
| <i>Trichoderma viride</i> | 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 |
| <i>Penicillium funiculosum</i> | 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 | 0.2 ^c | 0.25 ^c | 0.2 ^c | 0.5 ^f |
| <i>Penicillium ochrochloron</i> | 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 |
| <i>Penicillium verrucosum</i> | 0.11 ^b | 0.22 ^{bc} | 0.23 ^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.

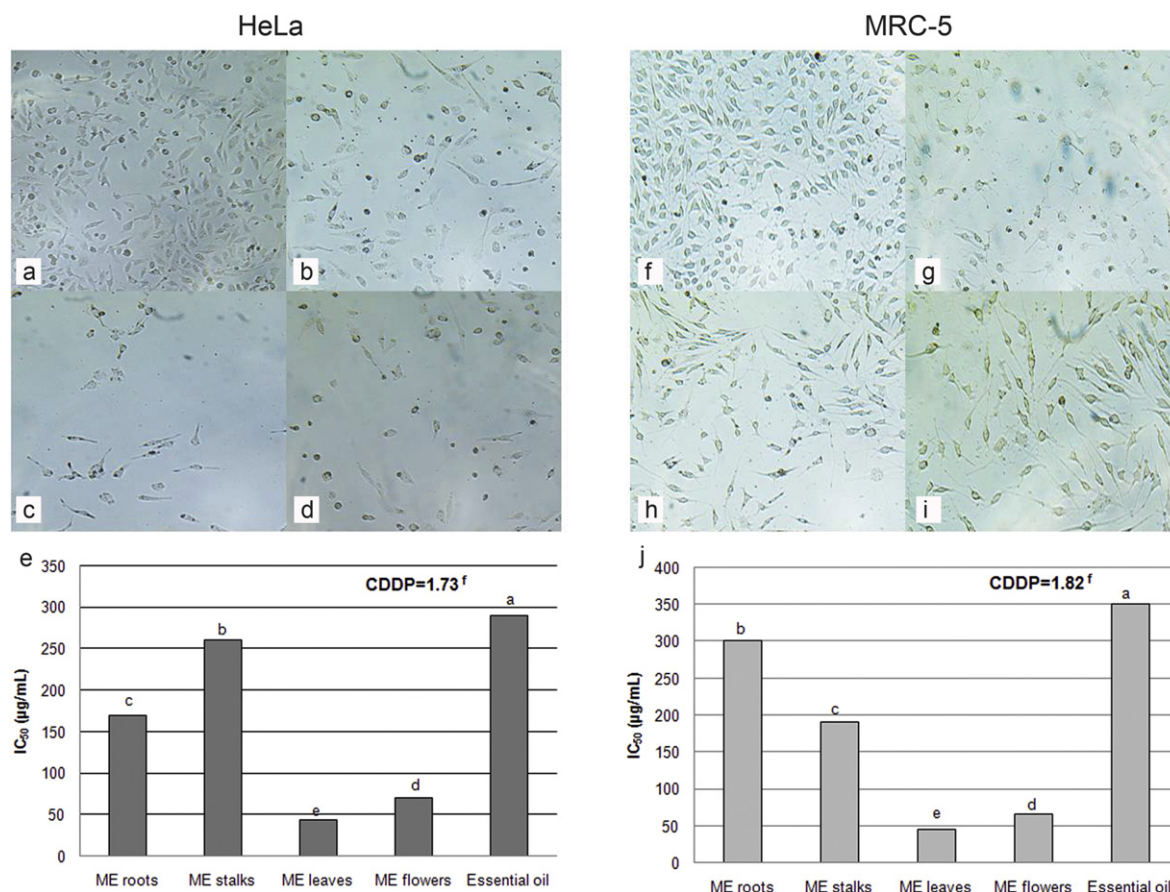


Fig. 2. Comparative presentation of photomicrographs and IC_{50} values ($\mu\text{g/mL}$), obtained, for 72 h of treatment in HeLa and MRC-5 cells. Photomicrographs present morphology of untreated HeLa cells (a), HeLa cells treated with: 1.7 μg CDDP (b), ME of leaves (c), ME of flowers (d). Morphology 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 ($\mu\text{g/mL}$), 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. funiculosus* 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*-dicafeoylquinic 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*-dicafeoylquinic and dicafeoylquinic 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. (Kähkönen et al., 1999; Wu et al., 2006; Juan-Badaturuge et al., 2009; Esmaeili et al., 2010; Barauskienė 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 streptomycin 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 IC₅₀, 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 IC₅₀ values up to 100 µg/mL. The leaves extract had the strongest antiproliferative 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.

Acknowledgements

The authors are grateful to the Ministry of Education, Science and Technological Development of Serbia for financial support (Grants Nos. OI173015, OI173032 and III41026).

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., Kazarnavičiūtė, R., Pukalskienė, M., Maždzierienė, R., Venskutonis, P.R., 2014. Agrofines 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.

Houghton, 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-Badaturge, M., Habtemariam, S., Jackson, C., Thomas, M.J.K., 2009. Antioxidant principles of *Tanacetum vulgare* L. aerial parts. *Natural Product Communications* 4, 1561–1564.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, J., 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., Buschiazzo, H.O., Mandrile, E., Rosella, M., Schinella, G., Fioravanti, D., 1996. Anti-inflammatory activity of *Tanacetum vulgare*. *Fitoterapia* 67, 319–322.

Móricz, M.A., Hábe, T.T., Bözörményi, 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.Á., 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 xanthosin 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.
- Scheerer, W.R., 1984. Components of oil of Tansy (*Tanacetum vulgare*) that repel Colorado potato beetles (*Leptinotarsa decemlineata*). *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., Hout, 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., Sodobekov, I.S., Akimayev, 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.