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Biological activity and essential oil composition of two new *Tanacetum chiliophyllum* (Fisch. & Mey.) Schultz Bip. var. *chiliophyllum* chemotypes from Turkey☆

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ABSTRACT

Water-distilled essential oils from aerial parts of *Tanacetum chiliophyllum* (Fisch. & Mey.) Schultz Bip. var. *chiliophyllum*, from two different localities in Turkey were analyzed by GC and GC/MS. The flower and stem oils of *T. chiliophyllum* var. *chiliophyllum* collected from Van-Muradiye location were characterized with camphor (32.5%, 36.2%), 1,8-cineole (1.6%, 16.1%), chamazulene (9.2%, 2.9%), for the first sample from this location and 1,8-cineole (12%, 18.4%), terpinene-4-o1 (10.3%, 9%), (*E*)-sesquilavandullol (5.8%, 1.6%), *p*-cymene (5.4%, 5.4%), hexadecanoic acid (4.2%, 7.6%) for the second plant sample. The flower and stem oils of the plant collected from Van-Güzeldere location were characterized with 1,8-cineole (22.1%, 28.9%), terpinene-4-ol (6.5%, 5.6%), α-pinene (5.3%, 1.5%). Five chemotypes were proposed according to biosynthetic origin of the main components of the investigated oils and previous investigations and they were tested with AHC analysis. Antibacterial activity of the oils were evaluated for five Gram-positive and five Gram-negative bacteria by using a broth microdulition assay. The highest activity was observed on *Escherichia coli* with the stem oil of the first sample from Muradiye (62.5 μg/mL) which gave same MIC with the positive control chloramphenicol. The highest DPPH scavenging activity was observed on the stem oil from the first sample of Muradiye location at 15 mg/mL concentration (79.1%). The oils showed moderate DPPH scavenging activity when compared with the positive control α-tocopherol. All of the oils except for flower and stem oils from the second sample of Muradiye location showed toxicity against *Vibrio fischeri* in the TLC-bioluminescence assay.

1. Introduction

Tanacetum chiliophyllum varieties are native plants of south west Asia including Turkey as well as north west of Iran, Azerbeijan and Armenia. This species is represented in Turkey with four varieties which are var. *monocephalum*, var. *oligocephalum*, var. *heimerlei* and var. *chiliophyllum* (Davis, 1975).

Tanacetum chiliophyllum var. *chiliophyllum* is known with "çeren", "ormadere" and "yavşan" local names in Eastern Anatolia (Altundag and Özhatay, 2009; Altundag and Ozturk, 2011). Decoction of the flowers heads of *T. chiliophyllum* var. *chiliophyllum* is used against pulmonic disorders, kidney stones and as antipyretic in traditional medicine (Altundag and Ozturk, 2011). It was worthy to note that in our field studies villagers reported snake deterrent properties of this plant however, to the best of our knowledge there is no report on the plant regarding such activity.

Various biological activities due to sesquiterpene lactone content of *Tanacetum* species are well known (Gören et al., 2002) however, to the best of our knowledge only report on the biological activity of the extracts of *T. chiliophyllum* varieties was on insecticidal activity and DPPH scavenging activity of *T. chiliophyllum* var. *monocephalum* extracts (Polatoğlu et al., 2011c).

Previous reports indicate the isolation of sesquiterpene lactone tamirin (Matsakanyan and Revazova, 1974), flavonoids scutellarein-6,7,4′-trimethyl ether, 6-hydroxyluteolin-6,3′4′-trimethyl ether, 6-hydroxyluteolin-6,3′-dimethyl ether, scutellarein-6,7-dimethyl ether and flavonols quercetagetin-3,6,4′-trimethyl ether, quercetagetin-3,6,7-trimethyl ether (Wollenweber et al., 1989) from *T. chiliophyllum* var. *chiliophyllum*.

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Also new sesquiterpene lactone type compounds chiliophyllin, heimerlein and known spiciformin, deacetyllauerenbiolide, 1α-hydroperoxy-1-desoxo chrysanolide, tabulin, tanachin, tamirin, dentatin, were isolated from *T. chiliophyllum* var. *heimerlei* (Gören and Tahtasakal, 1993, 1994) and 1-*epi*-chiliophyllin from *T. chiliophyllum* var. *monocephalum* (Polatoğlu et al., 2011c). Flavonoids and triterpenes 4′,5,7-trihydroxy-3′,8-dimethoxyflavone, 4′,5,7-trihydroxy-8-methoxyflavone, neolupenyl acetate (lup-12-ene-3β-acetate) were also reported from *T. chiliophyllum* var. *monocephalum* (Polatoğlu et al., 2011c). Biological activities and composition of the essential oil from *T. chiliophyllum* var. *chiliophyllum* growing in Erzurum and Elazığ locations were reported in the literature. The main essential oil components were reported as camphor 28.5%, 1,8-cineole 17.1%, camphene 7.1%, isobornyl propionate 5.4%, carveol 4.5%, 3-cyclohexen-l-ol 3.5% for the plant from Elazığ and camphor 17.9%, 1,8-cineole 16.6%, bomeol 15.4%, dihydro-α-cyclogeranyl hexanoate 10.1% and dihydro-α-cyclogeranyl pentanoate 3% for the plant from Erzurum (Bagci et al., 2008; Salamci et al., 2007). Also the essential oil composition of this plant from Bayburt location reported as camphor 16.8%, *cis*-chrysanthenyl acetate 16.3%, α-thujone 12.5% and nonadecane 3.6% (Başer et al., 2001). Previous reports show variation in the main components of *T. chiliophyllum* var. *chiliophyllum* essential oils. There are many examples of chemo variation in the essential oils at species level such as the examples for *T. vulgare*, *T. nubigenum* species (Chanotiya et al., 2005; Chanotiya and Mathela, 2007; Collin et al., 1993; Dev et al., 2001; Hendriks et al., 1990; Judzentiene and Mockute, 2004, 2005; Keskitalo et al., 2001; Mathela et al., 2008; Ognyanov et al., 1992; Rohloff et al., 2004). In addition variation of the oils obtained from subspecies (Judzentiene and Mockute, 2005; Polatoğlu et al., 2009a) and varieties (Bagci et al., 2008; Salamci et al., 2007) of genus *Tanacetum* have been also reported in recent years. In our ongoing research on phytochemical and biological investigation of this genus in Turkey (Polatoğlu et al., 2009a,b, 2010a,b,c, 2011a,b,c) here we report on the antibacterial, cytotoxic, DPPH scavenging activities and essential oil composition of *T. chiliophyllum* var. *chiliophyllum* from two different localities in East Anatolia region of Turkey that showed differences in their morphological properties and in their essential oil compositions.

2. Materials and methods

2.1. Plant materials

Plant materials were collected during the flowering period in 22–23 July 2006 from two different locations provinces Van-Muradiye at 2494 m above sea level and Van-Güzeldere at 2805 m above sea level. Two different sample groups were collected from the province Van-Muradiye since there were two groups of plants that distinctively showed morphological variations from each other and another sample group was collected from the province Van-Güzeldere. Voucher specimens have been deposited at the Herbarium of the Faculty of Science, Istanbul University (Voucher no. ISTE 83756, ISTE 85431 and ISTE 85430 respectively), Turkey. Plant materials were identified by Dr. Kerim Alpınar.

2.2. Isolation of the essential oils

Flowers and stems (100 g each) of the plant samples from province Van-Muradiye (A, B, C, and D) and Van-Güzeldere (E and F) locations were separately subjected to hydrodistillation for 4 h using a Clevenger-type apparatus to produce the oils. Second sample (C and D) from Muradiye with 68 g flowers and 100 g stems were subjected to same procedure. Blue colored oils were obtained from the first plant sample of Muradiye with 0.1% (A), 0.2% (B) (v/w) yields for flowers and stems respectively. Yellow colored oils were obtained from the second sample of Muradiye with 0.06% (C), 0.06% (D) (v/w) yields for flowers and stems respectively. Yellow colored oils were obtained from the sample of Güzeldere with 0.16% (E), 0.1% (F) (v/w) yields for flowers and stems respectively.

2.3. Gas chromatography–mass spectrometry analysis

The essential oil analysis were done simultaneously by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) systems.

The GC–MS analysis were done with an Agilent 5975 GC-MSD system with Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) and helium as carrier gas (0.8 mL/min). Oven temperature was programmed to 60 °C for 10 min and raised to 220 °C at rate of 4 °C/min. Temperature kept constant at 220 °C for 10 min and than raised to 240 °C at a rate of 1 °C/min. The injector temperature was set at 250 °C. Split flow was adjusted at 50:1. Mass spectra were recorded at 70 eV with the mass range m/z 35 to 450.

The GC analyses were done with Agilent 6890N GC system. FID detector temperature was set to 300 °C and same operational conditions applied to a duplicate of the same column used in GC–MS analyses. Simultaneous auto injection was done to obtain the same retention times. Relative percentage amounts of the separated compounds were calculated from integration of the peaks in FID chromatograms (Table 1).

Identification of essential oil components were done by comparison of their retention times with authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library) (Başer and Demirci, 2007) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (Jennings and Shibamoto, 1980; Joulain and König, 1998) was used for identification.

2.4. Antibacterial activity assay

Five Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermis* ATCC 12228, *Bacillus cereus* NRRL B-3711, *Bacillus subtilis* NRRL B-4378, Meticillin resistant *S. aureus* (Clinical isolate)) and five Gram-negative bacteria (*Escherichia coli* NRRL B-3008, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter aerogenes* NRRL 3567, *Proteus vulgaris* NRRL B-123, *Salmonella typhimurium* ATCC 13311) were used in this study. The minimum inhibitory concentration (MIC) values were determined for all of the oils, on each organism by using microplate dillution method (Iscan et al., 2002). Stock solutions of the oils (2 mg/mL) and standard antibacterial compound chloramphenicol (2 mg/mL) were prepared. Liquid medium was diluted by adding 25% DMSO or CH_3OH. Serial dilution was done on 96-well microlitre plates. Bacteria were standardized according to McFarland No: 0.5 after incubation 24 h at 37 °C on MHB. Cultures were mixed with essential oils and were incubated 24 h at 37 °C. Minimum inhibitory concentrations (MIC: μg/mL) were detected at the minimum concentration where bacterial growth was inhibited. 1% 2,3,5-Triphenyltetrazolium chloride (TTC, Aldrich, St. Louis, MO, USA) was used as an indicator of bacterial growth. Essential oil free solutions were used as blank controls and chloramphenicol was used as a positive control. All the experiments were performed in triplicate and means of results were given for the MIC values of the oils (Table 2).