

Supporting Information

Rec. Nat. Prod. 6:2 (2012) 184-188

Essential oil composition and antimicrobial activities of *Tanacetum chiliophyllum* (Fisch. & Mey.) Schultz Bip. var. *monocephalum* Grierson from Turkey

Kaan Polatođlu^{1,3,*}, Fatih Demirci², Betül Demirci², Nezhun Gören³ and
Kemal Hüsnü Can Başer⁴

¹Department of Pharmaceutical Analytical Chemistry, 10, Near East University, Mersin, Türkiye

²Department of Pharmacognosy, 26470, Anadolu University, Eskişehir, Türkiye

³Department of Biology, 34210, Yıldız Technical University, İstanbul, Türkiye

⁴Department of Botany and Microbiology, 11451, King Saud University, Riyadh, Saudi Arabia

Table of Contents	Page
S1: Gas Chromatography – Mass Spectrometry Analysis	2
S2: Antibacterial Activity Test	2
S3: <i>Vibrio fischeri</i> Toxicity Test	2
S4: Antibacterial (MIC = µg/mL) and cytotoxic activity of <i>T. chiliophyllum</i> var. <i>monocephalum</i> oils.	3
S5: References	3

* Corresponding author: E-mail: kaanpolatoglu@gmail.com

S1: Gas Chromatography-Mass Spectrometry Analysis: The essential oils were analyzed by GC and GC-MS simultaneously. GC-MS analyses were done with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μ m film thickness) was used with helium as carrier gas (0.8 mL/min). The injector temperature was set at 250°C and split ratio was adjusted at 40:1. 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 then raised to 240°C at a rate of 1 °C/min. Mass spectra were recorded at 70 eV with the mass range m/z 35 to 450. The GC analyses were carried out with Agilent 6890N GC system. FID detector temperature was set to 300°C. Same operational conditions applied to a duplicate of the same column used in GC-MS analyses. Simultaneous auto injection was carried out to obtain the same retention times. Relative percentage amounts of the separated compounds were calculated from integration of the peaks in FID chromatograms.

Identification of essential oil components was accomplished by comparison of their retention times with authentic samples or by comparison of their relative retention indices (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library) [1,2] 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 [3-5], was used for identification. Results of the analysis were given in Table 1.

S2: Antibacterial Activity test: 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. All of the microorganisms were developed on Mueller Hinton Agar (MHA, Merck Darmstadt, GERMANY) by incubation at 37°C. Microorganisms were freshly inoculated on liquid Mueller Hinton Broth (MHB, Merck Darmstadt, GERMANY) and incubated at the same temperature for 24 hours before use. The minimum inhibitory concentration (MIC) values were determined for flower and stem oils on each organism by using microplate dilution method [6]. Stock solutions (2 mg/mL) of the oils and standard antibacterial compound chloramphenicol were prepared. Liquid medium was used with adding 10-25% DMSO or Methanol. Serial dilution was done on 96-well microlitre plates. After growing bacteria 24 hours at 37°C on MHB they were adjusted according to McFarland No:0.5. Bacteria cultures were mixed with essential oils and were incubated 24 hours at 37°C. Minimum inhibitory concentrations (MIC: μ g/mL) were detected as the minimum concentration where microbial growth was missing. As an indicator of bacterial growth 1% 2,3,5-triphenyltetrazolium chloride (TTC, Aldrich St. Louis MO, USA) was used. Chloramphenicol was used as a positive control with pure solvent and essential oil free solutions as blank controls. Each experiment was performed in triplicate and means of results were given as MIC values of the oils in Table 2 in supporting information S4.

S3: Vibrio fischeri Toxicity: Bioluminescent, Gram-negative *Vibrio fischeri* (Chromadex™ Irvine CA, USA) marine bacteria was used for toxicity screening. HPTLC plates (Merck Darmstadt, GERMANY) were prepared with 5 μ L of 2 mg/mL ethanol solutions of the essential oils by Automatic TLC Sampler 4 (Camag Muttenz, Switzerland). Lyophilized *Vibrio fischeri* cultures were obtained from the kit. Luminescent *Vibrio fischeri* microorganisms were inoculated on the medium provided from the kit (Chromadex™ Irvine CA, USA). Culture of the microorganism was incubated for 24-30 hours at 28°C. Previously prepared HPTLC plates were dipped into the freshly grown luminescent culture with an automatic immersion device (Camag Muttenz, Switzerland) and excess of the culture removed from the plates with a squeegee. Plates were photographed at -30°C with CCD

camera of BioLuminizer (Camag Muttenz, Switzerland). Cytotoxicity of the oils were detected as black spots on the photographs [7].

S4: Table 2 - Antibacterial (MIC = µg/mL) and cytotoxic activity of *T. chiliophyllum* var. *monocephalum* oils.

Microorganism	A	B	+ C.
<i>Staphylococcus aureus</i>	>500 ^a	1000	62.5
Meticillin resistant <i>S. aureus</i>	125	500>	62.5
<i>Staphylococcus epidermis</i>	125	1000	31.2
<i>Bacillus cereus</i>	62.5	1000	125
<i>Bacillus subtilis</i>	250	1000>	62.5
<i>Escherichia coli</i>	≥500	>1000	62.5
<i>Pseudomonas aeruginosa</i>	500	1000	31.2
<i>Enterobacter aerogenes</i>	500>	>1000	62.5
<i>Proteus vulgaris</i>	500>	1000>	62.5
<i>Salmonella typhimurium</i>	500>	1000>	125
<i>Vibrio fischeri</i>	Toxic	Toxic	N.A.

+ C. :Positive Control (chloramphenicol); N.A. : Not Available; ^a : results are given a mean of three parallel experiments.

S5: References

- [1] F. W. McLafferty and D. B. Stauffer (1989). The Wiley/NBS Registry of Mass Spectral Data. J. Wiley and Sons, New York.
- [2] D. Joulain, W. A. König and D. H. Hochmuth (2001). Terpenoids and Related Constituents of Essential Oils. Library of MassFinder 2.1. Hamburg, Germany.
- [3] D. Joulain and W. A. König (1998). The Atlas of Spectra Data of Sesquiterpene Hydrocarbons. EB-Verlag, Hamburg.
- [4] ESO 2000 (1999). The Complete Database of Essential Oils, Boelens Aroma Chemical Information Service. The Netherlands.
- [5] W. G. Jennings and T. Shibamoto (1980). Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary GC. Academic Press, New York.
- [6] G. Iscan, N. Kirimer, M. Kürkcüoğlu, Kemal H. C. Baser and F. Demirci (2002). Antimicrobial screening: *Mentha piperita* essential oil, *J. Agric. Food Chem.* **50**, 3943-3946.
- [7] S. M. Verbitski, G. T. Gourdin, L. M. Ikenouye and J. D. McChesney (2008). Detection of *Actaea racemosa* adulteration by thin-layer chromatography combined thin-layer chromatography bioluminescence, *J. AOAC Int.* **91**, 2, 268-275.

