Supporting Information

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The Essential Oil Composition of *Tanacetum densum* (Labill.) Heywood ssp. *eginense* Heywood from Turkey

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S1: *Gas Chromatography-Mass Spectrometry Analysis:* The essential oil analyses were carried out simultaneously by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems. The oils were analyzed by GC using a HP 6890 system. An HP-Innowax FSC column (60 m x 0.25 mm Ø, with 0.25 μm film thickness) was used with nitrogen as carrier gas (1 mL/min). The oven temperature was kept at 60˚C for 10 min and programmed to 220˚C at a rate of 4˚C/min, then kept constant at 220˚C for 10 min and programmed to 240˚C at a rate of 1˚C/min. The injector temperature was set at 250˚C. The percentage compositions of the individual components were obtained from electronic integration measurements using flame ionization detection (FID, 250˚C). n-Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentages of the characterized components were given in Table 2. GC/MS analysis was performed with a Hewlett-Packard GCD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas. GC oven temperature conditions were as described above. Split flow was adjusted at 50 mL/min. The injector temperature was at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 425. Identification of essential oil components was carried out by comparison of their retention times with authentic samples or by comparison of their relative retention indices (RRI). 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.

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. The minimum inhibitory concentration (MIC) values were determined for all of the oils, on each organism by using microplate dilution method [6]. Stock solutions of the oils (2 mg/mL) and standart antibacterial compound chloramphenicol were prepared in the liquid medium Mueller Hinton Broth (MHB, containing 25% DMSO - for solubility enhancement of the oil). Serial dilution of the initial concentrations was prepared on 96-well microlitre plates containing equal amounts of distilled water. Bacterial suspension concentrations were standardized to McFarland No:0.5 after incubation 24 hours at 37˚C in MHB. Cultures were mixed with essential oils and were incubated 24 hours at 37˚C. Minimum inhibitory concentrations (MIC: μg/mL) were detected at the minimum concentration where bacterial growth was missing. 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 negative control 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. The results of antibacterial activity tests are given together with toxicity to *Vibrio fischeri* results in Table 3 which is given in supporting information **S5**.

S3: *Vibrio Fischeri Toxicity:* 5 μL of 2 mg/mL ethanol solutions of the essential oils were applied on HPTLC plates (Merck Darmstadt, GERMANY) by the help of Automatic TLC Sampler 4 (Camag Muttenz,Switzerland). Freeze-dried, luminescent Vibrio fischeri microorganisms obtained from the kit were inoculated on the medium provided by the kit (ChromadexTM 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]. The results of *Vibrio fischeri* toxicity activity test are given together with antibacterial activity results in Table 3 which is given in supporting information **S5**.

S4: Table 2. Comparison of essential oil components (≥3%) of *T. densum* subspecies.

Compound	**I**	**II**	**III**	**IV**	**V**	**VI**	**A**	**B**	**C**
Santolinatriene	3.5	0.9	-	5.0	-	-			
α-Pinene	2.3	3.3	5.0	0.7	3.0	9.7	7.0	3.1	5.3
Camphene	2.5	1.5	-	0.4	0.7	0.4	**10.6**	4.4	7.0
β- Pinene	2.2	2.3	0.1	0.5	0.8	**27.2**	1.6	0.8	1.3
α-Phellandrene	tr	-	-	-	-	3.2	-	-	-
α-Terpinene	0.3	0.2	-	3.9	-	0.1	0.2	tr	tr
p-Cymene	0.9	1.5	-	6.1	-	8.9	0.6	0.3	0.3
1,8-Cineole	**21.1**	**28.3**	1.5	**11.5**	**14.7**	**13.1**	**12.4**	3.2	2.9
Camphor	**19.2**	**16.4**	-	**15.6**	1.2	1.1	**30.9**	**25.7**	**27.7**
Isopinocarveol	-	-	0.8	-	3.3	-	-	-	-
Bornylacetate	1.1	1.2	-	-	-	0.3	4.3	9.4	**11.8**
Lavandulyl acetate	-	-	-	-	-	8.1	-	-	-
Borneol	5.8	6.4	1.6	7.5	**31.3**	1.2	3.6	5.1	5.2
Endoborneol	-	-	-	-	**21.0**	-	-	-	-
Neodihydro carveol	-	-	-	-	-	-	5.1	3.5	2.4
(+)-*epi*-Bicyclosesquiphellandrene	-	-	**31.4**	-	-	-	-	-	-
β-Patchoulene	-	-	-	**17.5**	-	-	-	-	-
α-Cadinol	tr	-	7.0	-	-	0.2	-	-	-
β-Selinene	-	-	0.2	5.0	-	-	-	-	-
1-Heptadecanol	-	-	5.6	-	-	0.1	-	-	-
Eicosane	-	-	3.2	-	0.1	-	-	-	-
Literature	**[9]**		**[10]**	**[8]**	**[10]**	**[11]**	**A**	**B**	**C**

Compounds were not given according to their retenition time order in the table. Relative percents of the compounds were given in the table. I: *T. densum* ssp. *sivasicum* – flower oil; **II:** *T. densum* ssp. *sivasicum* – stem oil; **III:** *T. densum* ssp. *laxum* – aerial parts oil; **IV:** *T. densum* ssp. *amani* – aerial parts oil; **V:** *T. densum* ssp. *amani* – aerial parts oil; **VI:** ***T. densum*** ssp. *amani* – basal leaves oil; **A:** *T. densum* ssp. *eginense* – flower oil; **B:** *T. densum* ssp. *eginense* – stem oil; **C:** *T. densum* ssp. *eginense* – basal leaves oil.

S5: Table 3. Antibacterial activity (MIC: µg/mL) and *Vibrio fischerii* cytotoxicity of A and B.

Microorganism	**Strain**	**A**	**B**	**Chloramphenicol**
Staphylococcus aureus	ATCC 6538	500	250	62.5
Meticillin resistant *S. aureus*	Clinical isolate	500	250	62.5
Staphylococcus epidermis	ATCC 12228	500>	125	31.2
Bacillus cereus	NRRL B-3711	500	500	125
Bacillus subtilis	NRRL B-4378	250	250	62.5
Escherichia coli	NRRL B-3008	500	>500	62.5
Pseudomonas aeruginosa	ATCC 27853	125	125	31.2

Enterobacter aerogenes	NRRL 3567	500	≥500	62.5
Salmonella typhimurium	ATCC 13311	500	500	125
Proteus vulgaris	NRRL B-123	500>	250	62.5
Vibrio fischeri		Toxic	Toxic	N.A.

Vitamin C is used as positive control in *Vibrio fischeri* cytotoxicity assay. A: *T. densum* ssp. *eginense* – flower oil; **B:** *T. densum* ssp. *eginense* – stem oil; **C:** *T. densum* ssp. *eginense* – basal leaf oil.

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