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Essential Oil Composition and Antimicrobial Activities of *Tanacetum chiliophyllum* (Fisch. & Mey.) Schultz Bip. var. *monocephalum* Grierson from Turkey

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Abstract: Water-distilled essential oils from aerial parts of *Tanacetum chiliophyllum* (Fisch. & Mey.) Schultz Bip. var. *monocephalum* Grierson from Turkey were analyzed by GC and GC/MS. The flower and stem oils were characterized by camphor (17.3%, 10.4%), 1,8-cineole (8.3%, 2.5%) and unknown compounds M^+ 218 (6.6%, 10.4%), M^+ 220 (Stem: 9.2%). Root oil was characterized with hexadecanoic acid (37.5%), alismol (6.3%), geranyl isovalerate (5.3%). Antibacterial activity of the flower and stem oils were evaluated on *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus vulgaris, Salmonella typhimurium, Staphylococcus epidermis, Bacillus cereus, Bacillus subtilis,* and Meticillin resistant *S. aureus* microorganisms by using a micro-dilution assay. Flower oil inhibited the growth of *Bacillus cereus* with the MIC 62.5 µg/mL which was 2 fold less concentration than the positive control chloramphenicol. Both flower and stem oils showed relative toxicity to *Vibrio fischeri* in the TLC-bioluminescence assay.

Keywords: Asteraceae*; Tanacetum chiliophyllum* var. *monocephalum*; essential oil; *Vibrio fischeri* TLC-bioluminescence cytotoxicity assay; antimicrobial activity; camphor; 1,8-cineole; hexadecanoic acid; alismol.

1. Plant Source

Tanacetum chiliophyllum (Fisch. & Mey.) Schultz Bip. var. *monocephalum* Grierson (Asteraceae) grows naturally in NorthWestern Iran, Azerbaijan, Armenia and East Turkey. Flowering time for this species is between May and June on volcanic, limestone slopes between 1200-3200 m altitude. *Tanacetum chiliophyllum* is represented in Turkey with four varieties which are var. *monocephalum*, var. *chiliophyllum*, var. *oligocephalum* and var. *heimerlei* [1]. Plant materials were collected in June 2006 from South East province of Van Turkey at 2954 m altitude. Plant material was identified by Dr. Kerim Alpınar and herbarium specimens have been deposited at the Herbarium of the Faculty of Pharmacy, Istanbul University with Voucher No: ISTE 83478

2. Previous Studies

Previous investigations reported essential oil composition of *T. chiliophyllum* var. *chiliophyllum* from different locations with different main components. According to these reports it seems there are three different chemotypes of this plant with camphor (28.5%), 1,8-cineole (17.1%), camphene (7.1%), isobornyl propionate (5.4%) [2]; camphor (17.9%), 1,8-cineole (16.6%), borneol (15.4%), dihydro-α-cyclogeranyl hexanoate (10.1%)

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[3] and camphor (16.8%), *cis*-chrysanthenyl acetate (16.3%), *α*-thujone (12.5%) as main constituents [4]. Chemovariation is a well known fact in *Tanacetum* species which is encountered on species level [5-7], and on subspecies level [8,9]. In our previous investigations on *Tanacetum* genus we have identified essential oil compositions of *T. cadmeum* ssp. *orientale* [8], *T. densum* ssp. *sivasicum, T. densum ssp. amani* [10,11], *T. macrophyllum* [12], *T. armenum*, *T. balsamita*, *T. haradjani*, *T. argyrophyllum* var. *argyrophyllum*, *T. argenteum* ssp. *argenteum* var. *canum*, *T. argenteum* ssp. *argenteum, T. praeteritum* ssp. *praeteritum*, and *T. praeteritum* ssp. *massicyticum*, *T. parthenium, T. zahlbruckneri, T. tabrisianum* [4,13-16]. Various biological activities and rich sesquiterpene content of *Tanacetum* extracts are well known [17]. Previous reports indicate isolation of sesquiterpene lactones from this species which include isolation of tamirin from *T. chiliophyllum* var. *chiliophyllum* [18]; new chiliophyllin, heimerlein and known spiciformin, deacetyllauerenbiolide, 1*α*-hydroperoxy-1-desoxo chrysanolide, tabulin, tanachin, tamirin, dentatin from *T. chiliophyllum* var. *heimerlei* [19,20]. To the best of our knowledge, except a report on the (+)-linalool content of its oil [21] there is no previous report on the phytochemistry, essential oil composition, antibacterial activity or cytotoxicity of *T. chiliophyllum* var. *monocephalum*. In the course of our biological activity screening project of *Tanacetum* species growing in Turkey, here we report on the essential oil compositions, antibacterial and cytotoxic activities of *T. chiliophyllum* var. *monocephalum* oils.

3. Present Study

Isolation of the Essential Oils: Flower, stem and roots (100g each) of the air dried plant samples were separately subjected to hydrodistillation for 4h using a Clevenger-type apparatus to obtain the oils. Yellow colored oil was obtained from each part of the plant with 0.06%, 0.05% (v/w) yields for flower and stem oils respectively. Root oil (<0.01%) was retrieved from the apparatus with n-hexane.

Gas Chromatography-Mass Spectrometry Analysis: Results of the analysis were given in Table 1. Method employed in the analysis was given in supporting information S1.

Antibacterial Activity test: Method employed in the tests was given in supporting information S2. Results of the antibacterial tests were given in Table 2 which was given in supporting information S4.

Vibrio Fischeri Toxicity: Method employed in the tests was given in supporting information S3. Results of the *Vibrion fischeri* toxicity tests were in Table 2 which was given in supporting information S4.

Composition of the oils were given in Table 1 with their relative percentages. The essential oils obtained from various parts of *T. chiliophyllum* var. *monocephalum* yielded oils with yellow color. A total of 93, 85 and 29 compounds were identified in flower, stem and root oils which represent 57.5%, 44.5% and 63.6% of the oils, respectively. Flower and stem oils were rich in camphor (17.3%, 10.4%) and 1,8-cineole (8.3%, 2.5%). Root oils were rich in hexadecanoic acid (37.5%), alismol (6.3%) and geranyl isovalerate (5.3%). All of the oils contained a couple of unidentified compounds with high percentages. The structure of these compounds could not be identified because of the low yield of the oils and inadequate amount of the plant material. Essential oils from *Tanacetum* species are commonly rich in 1,8-cineole, camphor, borneol, thujone, chrysanthenyl esters and alcohols [4,13]. In some species essential oils were found to be rich in carvone, pinenes and irregular monoterpenes such as lavandulyl esters and alcohol, artemisia ketone [22-24].

Table 1. Composition (%) of flower stem and root oils of *T. chiliophyllum* var. *monocephalum*. *(Continued overleaf)*

RI	Compound	Percent Composition		
		A	B	C
1014	Tricyclene	0.2	-	-
1032	*α*-Pinene	0.3	-	tr
1043	Santolinatriene	1.6	0.1	-
1076	Camphene	3.4	0.2	-
1093	Hexanal	0.1	-	-
1118	*β*-Pinene	0.1	-	-
1132	Sabinene	tr	tr	tr
1135	Thuja-2,4(10)-diene	0.1	-	-
1159	*δ*-3-Carene	-	-	tr
1194	Heptanal	tr	-	-
1195	Dehydro 1,8-cineole	tr	-	-
1213	1,8-Cineole	8.3	2.5	-
1255	*γ*-Terpinene	0.1	0.1	-

1280	*p*-Cymene	0.9	0.1	tr
1296	Octanal	0.1	-	-
1299	2-Methyl butyl isovalerate	0.1	0.1	-
1348	6-Methyl-5-heptene-2-one	-	tr	-
1400	Nonanal	0.1	0.1	-
1400	Tetradecane	tr	-	-
1403	Yomogi alcohol	0.1	-	-
1405	Santolina alcohol	0.6	-	-
1443	*α,p*-Dimethyl styrene	tr	-	-
1445	Filifolene	0.2	-	-
1465	Eucarvone	tr	-	-
1474	Camphenilone	0.1	-	-
1474	*trans*-Sabinene hydrate	-	0.7	-
1482	Longipinene*	0.4	0.4	-
1492	Cyclosativene	0.2	0.3	-
1497	*α*-Copaene	0.1	tr	-
1499	*α*-Campholene aldehyde	tr	-	-
1506	Decanal	-	tr	-
1532	Camphor	17.3	10.4	-
1535	*β*-Bourbonene	-	tr	-
1538	*trans*-Chrysanthenyl acetate	0.9	0.9	-
1541	Benzaldehyde	-	tr	-
1547	Dihydro achillene	0.1	tr	-
1553	Linalool	0.2	0.2	-
1556	*cis*-Sabinene hydrate	-	0.1	-
1562	Octanol	-	0.2	-
1571	*trans*-p-Menth-2-ene-1-ol	tr	0.1	-
1582	*cis*-Chrysanthenyl acetate	-	0.2	-
1583	Junipene (longifolene)	0.1	0.1	-
1586	Pinocarvone	1.4	1.3	-
1590	Bornyl acetate	0.3	0.2	-
1599	Chrysanthenyl propionate	0.5	0.4	-
1611	Terpinene-4-ol	1.3	0.9	-
1638	*cis-p*-Menth-2-ene-1-ol	tr	0.1	-
1643	Dehydrosabinaketone	tr	-	-
1648	Myrtenal	0.3	0.3	-
1651	Bornyl isobutyrate	-	0.2	-
1656	Chrysanthenyl isobutyrate	0.1	0.1	-
1657	Umbellulone	0.2	0.1	-
1668	(*Z*)-*β*-Farnesene	0.1	-	0.4
1669	Sesquisabinene	-	0.1	-
1670	*trans*-Pinocarveol	1.1	0.8	-
1682	*δ*-Terpineol	0.1	0.1	-
1683	*trans*-Verbenol	-	0.1	-
1684	*trans*-chrysanthemol	0.2	-	-
1685	Isovaleric acid	tr	-	-
1688	Selina-4,11-diene	-	tr	-
1689	*trans*-Piperitol (=*trans-p-Menth-1-en-3-ol*)	-	0.1	-
1694	Sylveterpineol	-	0.1	-
1700	1-Heptadecane	-	0.1	-
1704	Myrtenyl acetate	-	0.1	-
1704	*γ*-Muurolene	-	0.1	-
1706	*α*-Terpineol	0.3	-	-
1719	Borneol	2.9	1.2	-
1725	Verbenone	0.1	-	-
1726	Germacrene D	tr	tr	-
1740	*cis*-*α*-Bisabolene	-	0.2	-
1741	*β*-Bisabolene	-	-	1.7
1743	Chrysanthenyl isovalerate I	0.1	0.4	-
1751	Carvone	0.2	-	-
1758	*cis*-Piperitol	-	0.2	-
1760	Chrysanthenyl isovalerate II	0.3	0.3	-
1763	Naphthalene	0.1	0.1	0.5
1766	1-Decanol	-	tr	-
1773	*δ*-Cadinene	-	0.6	tr
1782	*cis*-carvyl acetate	-	0.2	-
1802	Cumin aldehyde	0.1	0.1	-
1804	Myrtenol	-	0.3	-
1808	Nerol	-	-	0.3
1819	(*E*)-2-Decen-1-ol	-	tr	-
1827	(*E,E*)-2,4-Decadienal	tr	0.1	tr
1838	(*E*)-*β*-Damascenone	0.1	-	-
1849	Calamenene	0.1	0.2	-
1857	Geraniol	0.1	0.1	-

1864	*p*-Cymen-8-ol	0.1	tr	-
1868	(*E*)-Geranyl acetone	0.1	-	0.4
1882	*α-ar*-himachalene	-	0.1	-
1889	*ar*-himachalene	0.1	-	-
1893	Geranyl isovalerate	-	0.3	5.3
1900	*epi*-Cubebol	0.3	0.6	tr
1900	Nonadecane	-	0.1	tr
1941	*α*-Calacorene	tr	0.1	-
1945	1,5-epoxysalvial-4(14)-ene	-	0.1	-
1957	Cubebol	-	1	tr
1958	(*E*)-*β*-Ionone	0.1	-	-
	Unknown I	6.6	10.4	2.6
2008	Caryophyllene oxide	0.6	0.4	0.6
2016	Isoamyl phenylacetate	-	0.1	-
2037	Salvial-4(14)-ene-1-one	0.1	0.1	0.3
2041	Pentadecanal	tr	-	-
2050	(*E*)-Nerolidol	tr	3.2	3.3
2073	*p*-Mentha-1,4-diene-7-ol	tr	-	-
2080	Cubenol	tr	tr	-
	Unknown II	-	9.2	2.2
	Unknown III	5.2	-	8.7
2098	Globulol	tr	-	-
2113	Cumin alcohol	0.3	-	-
2131	Hexahydrofarnesyl acetone	0.2	1.3	0.5
2144	Spathulenol	0.6	0.3	1.4
2148	Marsupellol	1.4	1	-
	Unknown IV	3	7.4	3.1
2183	*γ*-Decalactone	0.9	-	-
2186	Eugenol	-	-	0.6
2198	Thymol	-	0.3	-
2209	*T*-muurolol	0.2	-	-
2214	*ar*-Turmerol	0.2	-	-
2232	*α*-Bisabolol	-	1	0.4
2257	*β*-Eudesmol	0.6	0.3	-
2264	Alismol	-	0.4	6.3
2298	Decanoic acid	0.4	-	-
2300	Tricosane	0.5	-	-
2316	Caryophylladienol I	0.2	-	-
2369	Eudesm-4(15),7-dien-1*β*-ol	0.3	-	0.8
2400	Tetracosane	0.1	-	-
2500	Pentacosane	1	-	0.9
2607	1-Octadecanol	0.2	0.7	0.3
2622	Phytol	-	2.8	-
2670	Tetradecanoic acid	0.1	-	tr
2700	Heptacosane	0.7	0.5	-
2900	Nonacosane	tr	0.3	2.1
2931	Hexadecanoic acid	2.5	3.5	37.5
Monoterpene Hydrocarbons		7.4	0.9	0
Oxygenated Monoterpenes		37.8	23.4	6.6
Sesquiterpene Hydrocarbons		0.7	1.8	2.1
Oxygenated Sesquiterpenes		4.7	9.7	13.6
Others		6.9	8.7	41.3
Total Identified		**57.5**	**44.5**	**63.6**

t: trace; A: *T. chiliophyllum* var. *monocephalum* – Flower Oil; B: *T. chiliophyllum* var. *monocephalum* – Stem Oil; C: *T. chiliophyllum* var. *monocephalum* – Root Oil.
Unknown I EI/MS 70 ev *m/z* (rel. abun.) M^+ 218 (12), 203 (4), 190 (4), 175 (9), 161 (8), 147 (11), 132 (53), 125 (27), 119 (28), 107 (100), 91 (34), 77 (19), 67 (9), 55 (14), 41 (17)
Unknown II EI/MS 70 ev *m/z* (rel. abun.) M^+ 220 (14), 205 (5), 191 (4), 177 (30), 163 (17), 149 (28), 135 (24), 124 (95) 109 (100), 95 (80), 81 (97), 67 (55), 55 (43) 41 (48)
Unknown III EI/MS 70 ev *m/z* (rel. abun.) M^+ 222 (15), 204 (11), 189 (5), 178 (35), 159 (84), 147 (9), 134 (33), 119 (100), 108 (52), 93 (30), 81 (31), 71 (22), 56 (18), 43 (42)
Unknown IV EI/MS 70 ev *m/z* (rel. abun.) M^+ 220 (5), 206 (6), 187 (15), 177 (7), 159 (29), 145 (38), 132 (95), 119 (100), 107 (100), 91 (74), 79 (41), 67 (25), 55 (29), 41 (38)

The essential oils obtained from flowers and stems of *T. chiliophyllum* var. *monocephalum* inhibited all microorganisms at various MIC. Most significant inhibition was observed against *Bacilius cereus* with 62.5 µg/mL, which is found to be 2 fold less diluted than the positive control chloramphenicol (125 µg/mL) under the same test conditions. *B. cereus* is responsible for some of the food poisoning cases. It causes severe nausea, vomiting and diarrhea. Growth of this bacteria on food results in the production of enterotoxin causing the food poisoning [25,26]. Toxicity tests, activity guided isolation and structure elucidation studies are still required to understand the active principle in the oil and to find potential use for this oil with beneficial activity. For all other microorganisms MIC of positive control were lower than the oils. Both flower and stem oils showed toxicity to *Vibrio fischeri* which was observed as black spots on the TLC. This procedure was used to evaluate general toxicity of the oils as an initial indicator. The toxicity results observed at low concentrations when compared to Vitamin C which confirm that the oil inhibits the growth of *V. fischeri*. Results of antibacterial and cytotoxicity tests were given in Table 2 in supporting information **S4**.

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