SHORT REPORT

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Essential Oil Composition and Antibacterial Activity of *Tanacetum hololeucum* from Iran

Hamed Shamkhani[1], Naser Nasiri[1], Atousa Aliahmadi[2] and Ali Sonboli[*2]

[1] *Islamic Azad University, Pharmaceutical Sciences Branch, Tehran, Iran*
[2] *Department of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C., Evin, Tehran, Iran*

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Abstract: Composition and antibacterial activity of the hydro-distilled essential oil from aerial parts of *Tanacetum hololeucum* from Iran were investigated. The oil of *T. hololeucum* was analyzed by GC-FID and GC-MS. The major compounds were found to be artemisia alcohol (22.8%), yomogi alcohol (19.4%), artemisyl acetate (12.9%), γ-eudesmol (12.1%) and camphor (10.5%). The antibacterial activity of the oil was tested against four bacteria (*Staphylococcus aureus, Bacillus cereus, Klebsiella Pneumoniae* and *Escherichia coli*) by disk diffusion and determination of MIC and MBC values. The results showed that Gram-positive bacteria were highly inhibited by the oil with MIC values of 2 mg/mL. The most resistant strain was *Klebsiella Pneumoniae* with the highest MIC and MBC values of 16 and >32 mg/mL, respectively. Considering sensitivity screening it is notable that the antibacterial property of the oil from *T. hololeucum* could be attributed mainly to the synergistic property of main compounds.

Keywords: *Tanacetum*; Hydro-distillation; Yomogi alcohol; Artemisia alcohol; Iran.

1. Plant Source

The genus *Tanacetum* (Asteraceae, Anthemideae), is represented in Iran by 36 species, of which 18 are endemic [1]. *Tanacetum hololeucum* (Bornm.) Podl. is a small sized (8 – 20 cm) perennial aromatic herb growing in cracks on limestone slopes or screes. This species is endemic to northern parts of Iran, Alborz Mountain [2].

The aerial parts of *T. hololeucum*, collected at the full flowering stage from Damavand Mountain, Lar valley, Iran, and dried at ambient temperature. A voucher specimen (MPH - 1250) is deposited at the Herbarium of the Medicinal Plants and Drugs Research Institute, Shahid Beheshti University of Tehran, Iran.

2. Previous Studies

Tanacetum species generally have essential oils with a high content of camphor, 1,8-cineole, chrysanthenyl alcohols and esters, thujone and borneol [3–15]. Essential oil composition and antioxidant activities of the various extracts of *T. sonbolii* [3] and antimicrobial property of the

[*] Corresponding author. E-mail: a-sonboli@sbu.ac.ir , Phone, +98-21-22431783.

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essential oil of *T. fisherae* both from Iran [5] have been investigated. Essential oil composition and antimicrobial activities of *T. chiliophyllum* var. *monocephalum* [12] and two chemotypes of *T. chiliophyllum* var. *chiliophyllum* both from Turkey have been studied [13]. The essential oil of endemic *Tanacetum mucroniferum* from Turkey showed low DPPH scavenging activity [14].

3. Present Study

The powdered aerial flowering plant parts (50 g) were hydrodistilled using a Clevenger type apparatus for 3 h. The resulting essential oil was dried over anhydrous sodium sulfate and stored at 4°C until analyzed and tested.

***Essential oil analysis*:** GC-FID analyses of the oil were conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at a constant flow of 1.1 mL/min. The split ratio was 1/50. The oven temperature was raised from 60 ºC to 250 ºC at a rate of 5 ºC/min. The injector and detector (FID) temperatures were kept at 250 ºC and 280 ºC, respectively. GC-MS analysis was carried out on a Thermoquest-Finnigan Trace GC-MS instrument equipped with the same column and using the same temperature programming as mentioned for GC. Transfer line temperature was 250 ºC. Helium was used as the carrier gas at a flow rate of 1.1 mL/min, with a split ratio equal to 1/50. The constituents of the essential oils were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes ($C_6 - C_{24}$) and the oil on a DB-5 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectral library (Wiley 7.0) or with authentic compounds.

Antibacterial assay: In vitro antibacterial activity of the essential oil was assessed against two Gram-positive: *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (PTCC 1015) as well two Gram-negative bacteria; *Klebsiella Pneumoniae* (Clinical isolate) and *Escherichia coli* (ATCC 25922). Disk diffusion method was carried out using kirby-bauer method and according to the CLSI (Clinical Laboratory and Standards Institute) guideline [16]. Broth micro-dilution susceptibility method using 96 well trays was performed to determine the minimum concentration of essential oil required for inhibition of visible growth (MIC) or killing (MBC) of the test strains.

For this purpose standard protocol of CLSI (Clinical Laboratory and Standards Institute) was used with some modifications [16]. The inoculants of the bacterial strains were prepared from freshly cultured strains, by using sterile normal saline which were adjusted to 0.5 McFarland standard turbidity and then were further diluted (1:1000 for bacteria) by sterile Mueller-Hinton broth (MHB) just before adding to the wells containing a desired range of diluted essential oil. Essential oil was assessed in a concentration range from 64 to 0.06 mg/mL in wells containing MHB medium supplemented by 0.5% Tween-80 [17]. Inoculated trays were incubated for 20 h at 37°C and then the MICs were recorded as the lowest concentrations that could inhibit the visible growth of microorganisms. Minimum bactericidal concentration (MBC) was determined by sub-culturing of 100 μl medium from each negative well and from the positive growth control onto Nutrient agar plates. MBCs were defined as the lowest concentration that could kill 99.9% of the assessed microorganisms. Cefixime was used as standard antibiotic. All tests were performed in triplicate.

The hydrodistillation of aerial parts of *T. hololeucum* gave yellow oil in 0.3 % (w/w) yield, based on the dry weight of the plant. Thirty-five components were identified representing 99.9 % of the total oil. The qualitative and quantitative essential oil compositions are presented in Table 1, where compounds are listed in order of their elution on the DB – 5 column. GC-MS chromatogram of the essential oil of *T. hololeucum* is presented as supporting file (S1). The major constituents of the oil were found to be artemisia alcohol (22.8%), yomogi alcohol (19.4%), artemisyl acetate (12.9%), γ-eudesmol (12.1%) and camphor (10.5%) followed by *β*-pinene (3.6%), *α*-pinene (2.5%), neryl acetate (2.3%), *cis*-thujone (2.2%) and 1,8-cineole (2.1%). The classification of the identified constituents based on functional groups is summarized at the end of Table 1. The oxygenated monoterpenes as the principal compound group constituting 75.9% followed by oxygenated sesquiterpenes comprising 14.6% of the total oil. Monoterpene and sesquiterpene hydrocarbons represented 8.8 and 0.6 % of the oil, respectively.

Table 1. Percentage composition of the essential oil of *Tanacetum hololeucum*

No.	Component	RI[a]	LRI[b]	%
1	santolina triene	907	906	1.1
2	artemisia triene	925	923	0.2
3	α-pinene	941	932	2.5
4	camphene	958	946	0.8
5	sabinene	979	969	0.1
6	β-pinene	986	974	3.6
7	**yomogi alcohol**	**998**	**999**	**19.4**
8	*p*-cymene	1029	1020	0.3
9	limonene	1035	1024	0.1
10	1,8-cineole	1039	1026	2.1
11	artemisia ketone	1060	1056	0.4
12	γ-terpinene	1063	1062	0.1
13	*cis*-sabinene hydrate	1073	1065	0.2
14	**artemisia alcohol**	**1087**	**1080**	**22.8**
15	*cis*-thujone	1113	1101	2.2
16	*trnas*-thujone	1123	1112	0.6
17	**camphor**	**1156**	**1141**	**10.5**
18	**artemisyl acetate**	**1169**	**1169**	**12.9**
19	terpinen-4-ol	1186	1174	0.4
20	α-terpineol	1196	1186	0.1
21	myrtenal	1204	1195	0.2
22	*trans*-chrysanthenyl acetate	1244	1235	1.6
23	bornyl acetate	1293	1284	0.2
24	neryl acetate	1361	1359	2.3
25	α-copaene	1391	1374	0.1
26	(*E*)-caryophyllene	1440	1417	0.2
27	β-selinene	1492	1489	0.2
28	germacrene D	1500	1499	0.1
29	lavandulyl isovalerate	1508	1509	0.3
30	elemol	1564	1548	0.1
31	spathulenol	1598	1577	0.3
32	caryophyllene oxide	1608	1582	0.5
33	**γ-eudesmol**	**1656**	**1630**	**12.1**
34	β-eudesmol	1677	1649	0.6
35	valeranone	1701	1674	0.7
	Monoterpen hydrocarbons			8.8
	Oxygenated monoterpens			75.9
	Sesquiterpene hydrocarbons			0.6
	Oxygenated Sesquiterpens			14.6
	Total			**99.9**

[a] RI, retention indices relative to C_6–C_{24} *n*-alkanes on the DB-5 column;
[b] LRI, retention indices published in literature.

Once, Hayashi et al. [18] isolated and elucidated the structure of yomogi alcohol A, a monoterpene alcohol from *Artemisia feddei* Lev. et Van. Subsequently, this compound has been identified and reported in several species of genera *Artemisia* and *Achillea*. Yomogi alcohol (38.47%), artemisyl acetate (24.88%), and artemisia alcohol (6.70%) have already characterized as the dominant compounds in the essential oil of *Artemisia abyssinica* from Ethiopia [19]. In the essential oil obtained from *Artemisia lavandulaefolia* DC. from South Korea yomogi alcohol (4.5%) was found as one of the main compounds [20]. The major components of the leaf oil of *A. douglasiana* [21] have been found to be camphor (29%), artemisia ketone (26%) and artemisia alcohol (13%). As far as our literature survey could ascertain, these irregular monoterpenoids have not been reported in the essential oils of *Tanacetum* species.

Camphor, 1,8-cineole and borneol has almost been reported as the third major compounds in the essential oils of different *Tanacetum* species. A summary of the previous published data on the three main compounds of essential oils in *Tanacetum* species has been presented in Table 2. Regarding major compounds of *T. hololeucum* and previous reports on the oil compounds of other *Tanacetum* species (Table 2), it could be concluded that there is notable differences in the main marker constituents of the essential oils within *Tanacetum* species.

Table 2. A summary of the published data on the main compounds of *Tanacetum* essential oils

Species	Plant part	Camphor	1,8-Cineole	Borneol	Ref.
T. chiliophyllum var. *monocephalum*	FL	17.3%	8.3%	-	12
T. polycephalum subsp. *argyrophyllum*	AP	52.0%	36.3%	-	6
T. polycephalum subsp. *polycephalum*	AP	37.3%	8.8%	9.4%	6
T. polycephalum subsp. *duderanum*	AP	8.0%	-	8.3%	6
T. densum	FL	19.2%	21.1%	5.8%	15
	ST	16.4%	28.3%	6.4%	15
T. densum subsp. *amani*	AP	15.6%	11.5%	7.5%	10
	ST	10.4%	2.5%	-	10
T. cadmeum	FL	25.9%	6.6%	15.4%	8
	ST	14.8%	7.4%	25.8%	8
T. alyssifolium	AP	12.4%	4.8%	35.2%	7
T. parthenium	FL& ST	18.94%	-	10.93%	23
T. aucheranum	AP	11.6%	23.8%	3.8%	25
T. gracile	AP	-	15.2%	6.1%	29
T. tabrisianum	FL	1.4%	17.6%	6.9%	11
	ST	0.8%	22.5%	3.3%	11

AP, Arial Part; FL, Flower; ST, Stem

As shown in the table 3, assessed essential oil has revealed a moderate antibacterial activity. *S. aureus* has been the most sensitive bacterium together with *B. cereus* strain. However IZ of *S. aureus* has been greater than that of *B. cereus*. Both Gram-positive bacteria were inhibited by the essential oil with MIC values of 2 mg/mL whereas MIC values for *E. coli* and *K. pneumoniae* were 16 mg/mL. The lowest concentration which could result in fully inhibition by killing of microorganisms was that of *B. cereus* (MBC: 2 mg/mL). Tested *K. pneumoniae* strain was the most resistant bacterium with MIC value greater than 32 mg/mL.

Plants are known as hopeful and valuable natural resource of antimicrobial agents [22]. Antibacterial activities of the essential oils of different *Tanacetum* species have been reported previously [9,10, 12, 23] and biological activities of members of this genus have been reviewed by Kumar & Tyagi [24]. A moderate inhibitory activity for essential oils extracted from *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum* against 30 phytopathogenic fungi as well as 33 plant and human pathogenic bacteria has been reported by Salamci et al. [25]. They showed a broad spectrum of activities against both Gram positive and Gram negative bacteria. However in other study [10], assessed Gram positive bacteria such as *B. cereus* showed more sensitivity against essential oil of *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum* which is in accordance with the result of our study on *Tanacetum hololeucum*. Some constituents of assessed essential oil, such as camphor [26], *β*-pinene and *α*-pinene [27], and 1,8-cineole [26, 28] have a confirmed antibacterial properties. However the antibacterial property of *T. hololeucum* oil could be attributed mainly to the synergistic property of its main components.

Table 3. Antibacterial activity of the essential oil of *Tanacetum hololeucum*

Microorganisms	Essential oil			Cefexime	
	IZ[1]	MIC[2]	MBC[2]	MIC[3]	MBC[3]
Staphylococcus aureus (ATCC 25923)	35	2	32	1	4
Bacillus cereus (PTCC 1015)	14	2	4	0.5	1
Escherichia coli (ATCC 25922)	12	16	16	8	16
Klebsiella pneumoniae (Clinical isolate)	9	16	>32	32	64

[1] Inhibition zone diameter (mm); [2] Values given as mg/mL; [3] Values given as µg/mL.

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