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Essential Oil Composition and Antibacterial Activity of *Tanacetum argenteum* (Lam.) Willd. ssp. *argenteum* and *T. densum* (Lab.) Schultz Bip. ssp. *amani* Heywood from Turkey

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Abstract: Water-distilled essential oils from aerial parts of *Tanacetum argenteum* ssp. *argenteum* and *T. densum* ssp. *amani* from Turkey were analyzed by GC and GC/MS. The essential oil of *T. argenteum* ssp. *argenteum* was characterized with α-pinene 36.7%, β-pinene 27.5% and 1,8-cineole 9.8%. *T. densum* ssp. *amani* was characterized with β-pinene 27.2%, 1,8-cineole 13.1%, α-pinene 9.7% and *p*-cymene 8.9%. Antibacterial activity of the oils were evaluated for five Gram-positive and five Gram-negative bacteria by using a broth microdilution assay. The highest inhibitory activity was observed against *Bacillus cereus* for *T. argenteum* ssp. *argenteum* oil (125 μg/mL) when compared with positive control chloramphenicol it showed the same inhibition potency. However, the same oil showed lower inhibitory activity against *B. subtilis* when compared. The oil of *T. densum* ssp. *amani* did not show significant activity against the tested microorganisms. DPPH radical scavenging activity of the *T. argenteum* ssp. *argenteum* oil was investigated for 15 and 10 mg/mL concentrations. However, the oil did not show significant activity when compared to positive control α-tocopherol. Both oils showed toxicity to *Vibrio fischeri* in the TLC-bioluminescence assay.

Key words: *Tanacetum argenteum* ssp. *argenteum*, *T. densum* ssp. *amani*, Asteraceae, essential oils, α-pinene, β-pinene, 1,8-cineole, DPPH radical scavenging, antibacterial activity, *Vibrio fischeri* toxicity.

1 INTRODUCTION

Tanacetum argenteum is represented in Turkey with three subspecies; one of the subspecies is also represented with two varieties. Three of these taxa are endemic in Turkey including *T. argenteum* ssp. *argenteum*[1]. Previously essential oil composition of this species[2,3] and chemistry[4-6] of ssp. *flabellifolium* and ssp. *canum* var. *canum* were investigated. Also there is a report on the chemistry of ssp. *argentum*[7]. New sesquiterpene lactones 8α-angeloyloxycostunolide, 8α-angeloyloxyanhydroverlotorin[7], flabellin[4], 1β-hydroxy-6α-angeloyloxygermacra-4(5), 10(14), 11(13)-trien-8, 12-ollide, 1β,4α-dihydroxy-6α-isobutyloxyeudesm-11(13)-ene-8,12-ollide[5] epoxyflabellin, $\Delta^{3(4)}$-15-oxoflabellin, $\Delta^{3(4)}$-15-hydroxydihydroflabellin, 11α-dihydroflabellin and 11β-dihydroflabellin[6] were isolated from *T. argenteum* subspecies. However to the best of our knowledge there is no report on the essential oil composition of *T. argenteum* ssp. *argenteum*.

Tanacetum densum is represented with four subspecies all are endemic in Turkey. Previous investigations on this species include essential oil composition[8, 9] and chemistry[10-15] of ssp. *amani*, ssp. *eginense* and ssp. *sivasicum*.

As a part of our phytochemical and biological investigation of *Tanacetum* species, here we report on the composition and antibacterial, cytotoxic, radical scavenging properties of endemic *T. argenteum* ssp. *argenteum* and *T. densum* ssp. *amani* essential oils from Turkey.

2 EXPERIMENTAL

2.1 Plant materials

Plant materials *T. argenteum* ssp. *argenteum* (A) and *T. densum* ssp. *amani* (B) were collected during the flower-

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ing period in 14 July 2005 from Saimbeyli – Adana at 2107 m altitude. Voucher specimens have been deposited at the Herbarium of the Faculty of Science, Istanbul University (Voucher no. ISTE 83398 (A) and ISTE 83399 (B)), Turkey. Plant materials were identified by Dr. Kerim Alpınar.

2.2 Methods

2.2.1 Isolation of the essential oils

Aerial parts (100 g each) of the plant samples A and B from Saimbeyli location were separately subjected to hydrodistillation for 4 h using a Clevenger- type apparatus to produce the oils. Yellow colored oils were obtained in 0.27% (A) and 0.45% (B) (w/w) yields.

2.2.2 Essential oil analysis

The essential oil analyses were carried out simultaneously by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems.

2.2.2.1 Gas chromatography-mass spectrometry analysis

The GC-MS analysis was performed 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℃ for 10 min. and raised to 220℃ at rate of 4℃/min. Temperature kept constant at 220℃ for 10 min. and then raised to 240℃ at a rate of 1℃/min. Mass spectra were recorded at 70 eV with the mass range *m/z* 35 to 450.

2.2.2.2 Gas chromatography analysis

The GC analyses were done with an Agilent 6890N GC system. FID detector temperature was set to 300℃ 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. The result of analysis is shown in Table 1.

2.2.2.3 Identification of components

Identification of essential oil components were carried out 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)[16, 17] 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[18-20] was used for identification.

2.2.3 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 dilution method[21]. 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 h at 37℃ in MHB. Cultures were mixed with essential oils and were incubated 24 h at 37℃. 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 test are given together with *Vibrio fischeri* toxicity in Table 3.

2.2.4 *Vibrio fischeri* cytotoxicity assay

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 h at 28℃. 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℃ with CCD camera of BioLuminizer (Camag Muttenz,Switzerland). Cytotoxicity of the oils were detected as black spots on the photographs[22]. The results of *Vibrio fischeri* toxicity activity test are given together with antibacterial activity results in Table 3.

2.2.5 DPPH radical scavenging activity assay

Antioxidant activity of oil A was determined with DPPH radical protocol[23]. A modified protocol for HPTLC-DPPH[24] was used. Stock solutions of the oil A (10 and 15 mg/mL), positive control α-tocopherol (Aldrich, St. Louis MO, USA) (10 and 15 mg/mL) and DPPH (0.1 mM) (Aldrich, St. Louis MO, USA) were prepared with CH_3OH. 200 μL of the oil solutions were mixed with 1000 μL of DPPH solution as well as positive controls and essential oil free blank controls in 1.5 mL Eppendorf tubes and vortexed for 2 min. After incubating all the samples and controls for 1 h in dark at room temperature, 2 μL of them were applied on an aluminium 60 F254 TLC Plate (Merck Darmstadt, GERMANY) with 5 mm band length by the help of Linomat 5 TLC appli-

cator system (Camag Muttenz, Switzerland). After preparing samples and controls on the TLC; plates were scanned at 517 nm with a TLC Scanner 3 (Camag Muttenz, Switzerland) and absorbance of the bands were detected. Percent of DPPH scavenging property was calculated according to% DPPH Scav. Prop. $= [(A_{Control} - A_{Sample})/A_{Control}] \times 100$ formula. The results of antioxidant property activity test is given in Table 4.

DPPH radical scavenging activity was observed on the oil A with 15 mg/mL and 10 mg/mL concentration. The oil A showed very low DPPH scavenging activity when compared to the positive control α-tocopherol in both concentrations.

3 RESULTS

Essential oil compositions of *T. argenteum* ssp. *argenteum* (A) and *T. densum* ssp. *amani* (B) are given in Table 1. Ninety seven compounds were identified representing 95.3% of *T. argenteum* ssp. *argenteum* (A) oil. α-Pinene 36.7%, β-pinene 27.5% and 1,8-cineole 9.8% were found to be main components of the oil A. Eighty eight compounds were identified representing 90.7% of *T. densum* ssp. *amani* (B) oil. β-Pinene 27.2%, 1,8-cineole 13.1%, α-pinene 9.7% and *p*-cymene 8.9% are found to be main components of oil B. Previous reports indicate similar compositions with pinane type monoterpenes and 1,8-cineole as main components only for *T. vulgare*[25]. However essential oil profile of this oil is different than the previously reported oils of Tanacetum species. Previously reported *T. argenteum* ssp. *flabellifolium* oil also had a high content of α-pinene but it did not contain β-pinene and 1,8-cineole in high amounts[2]. Similarly *T. argenteum* ssp. *canum* var. *canum* essential oil differs from oil A with its high content of caryophyllene oxide and α-thujone[3]. A report on *T. densum* ssp. *amani* indicates its essential oil composition as β-patchoulene, camphor and 1,8-cineole[8] unlike the oil B. Another subspecies of *T. densum* contained high content of 1,8-cineole and camphor in its oils[9]. Comparison of main essential oil components of *T. argenteum* and *T. densum* subspecies cited from previous reports together with the present data are given in Table 2.

T. argenteum ssp. *argenteum* oil showed the same level of activity against the food pathogenic bacteria *Bacillus cereus* (125 μg/mL) when compared to the positive control chloramphenicol (125 μg/mL). Similarly oil A showed relatively mild inhibitory activity against *B. subtilis* (125 μg/mL) when compared to the positive control (62.5 μg/mL). However *T. densum* ssp. *amani* oil did not show any significant activity to the microorganisms tested in this series suggesting its selectivity. *B. cereus* is a food pathogen which is responsible for some of the food poisoning diseases by its ability to produce enterotoxin[26, 27]. Both oils showed toxicity to *Vibrio fischeri* with HPTLC-*Vibrio fischeri* toxicity assay which is used to evaluate possible general toxicity of the oils as an initial indicator. The toxicity was observed at low concentrations when compared to vitamin C.

4 DISCUSSION

Previously essential oils with high content of α-pinene were reported for *Tanacetum* species however to the best of our knowledge there is no previous report on the oil of this species with high content of both α-pinene and β-pinene. *T. argenteum* ssp. *argenteum* essential oil was investigated for the first time and it showed an unusual essential oil composition when compared to the other species of *Tanacetum*. On the other hand essential oil of *T. densum* ssp. *amani* was previously investigated and reported to have β-patchoulene, camphor, 1,8-cineole compounds in high amounts[8] unlike our present investigation on this plant. These differences in the previous literature and present data could be related to different collection times, climatic and soil conditions, ecological factors, methods and instruments employed in analysis or different genotypes. However to the best of our knowledge there is no report on *Tanacetum* essential oil with high content of β-patchoulene except for the previous report on *T. densum* ssp. *amani*[8]. This compound could have been mistaken with α-copaene or another component which has the similar mass fragmentation pattern and chromatographic properties with β-patchoulene. Main source for this compound is known as *Pogostemon* (Lamiaceae) species and essential oil of these species finds use in perfume industry. It is highly unlikely for this sesquiterpene to occur in a *Tanacetum* oil; however α-copaene is present in many *Tanacetum* essential oils as a satellite component[3,9,28-32]. In the previous literature pinane type monoterpenes were reported in small amounts however in this study both α-pinene and β-pinene were the main components. Also in the present data camphor is present in small amounts unlike the previous report. Both oils contained 1,8-cineole and *p*-cymene in similar amounts. However β-patchoulene is completely missing from the oils studied while α-copaene is present in trace amount. Oil A showed insignificant DPPH-scavenging activity. The same oil showed similar activity against *Bacillus cereus* when compared with positive control at the same concentration. Oil B did not show any significant activity to the tested microorganisms. Both oils showed toxicity to *Vibrio fischeri* when compared to positive control Vitamin C.

5 CONCLUSIONS

Essential oil compositions of *T. argenteum* ssp. *argen-*

Table 1 Essential Oil Composition of *T. argenteum* ssp. *argenteum* (A) and *T. densum* ssp. *amani* (B) Leaves.

RRI	Compound	A (%)	B (%)
1014	Tricyclene	tr	tr
1032	*α*-Pinene	36.7	9.7
1035	*α*-Thujane	1.2	0.6
1072	*α*-Fenchene	tr	−
1076	Camphene	0.2	0.4
1093	Hexanal	−	tr
1118	*β*-Pinene	27.5	27.2
1132	Sabinene	1.5	0.7
1135	Thuja-2,4(10)-diene	0.1	tr
1176	*α*-Phellandrene	0.7	3.2
1188	*α*-Terpinene	0.3	0.1
1194	Heptanal	tr	tr
1195	Dehydro 1,8-cineole	−	tr
1203	Limonene	1.5	0.7
1213	1,8-Cineole	9.8	13.1
1255	*γ*-Terpinene	0.1	0.2
1280	*p*-Cymene	2.1	8.9
1290	Terpinolene	0.2	0.1
1296	Octanal	tr	−
1303	Amyl isovalerate	−	tr
1385	Heptyl acetate	−	tr
1386	1-Octenyl acetate	−	tr
1386	*n*-Hexyl pivalate	−	tr
1400	Nonanal	tr	tr
1452	1-Octen-3-ol	−	0.2
1463	1-Heptanol	−	tr
1466	*α*-cubebene	0.1	−
1474	*trans*-Sabinene hydrate	−	0.5
1479	Linalool-7-oxide-3-one	0.6	−
1483	Octyl acetate	tr	−
1493	*α*-Ylangene	tr	−
1497	*α*-Copaene	0.1	tr
1499	*α*-Campholene aldehyde	tr	tr
1506	Decanal	tr	−
1532	Camphor	tr	1.1
1535	*β*-Bourbonene	0.1	−
1538	*trans*-Chrysanthenyl acetate	0.1	0.8
1541	Benzaldehyde	−	tr
1544	*α*-Gurjunene	−	tr
1553	Linalool	0.4	tr
1556	*cis*-Sabinene hydrate	tr	0.4
1568	1-Methyl-4-acetylcyclohex-1-ene	0.1	1.9
1571	*trans*-*p*-Menth-2-ene-1-ol	0.1	0.1
1586	Pinocarvone	0.1	0.2
1591	Bornyl acetate	−	0.3
1597	*β*-Copaene	tr	−
1611	Terpinene-4-ol	0.8	0.6
1617	Lavandulyl acetate	tr	8.1
1638	*cis*-*p*-Menth-2-ene-1-ol	tr	0.1
1639	Cadina-3,5-diene	tr	−
1648	Myrtenal	0.1	0.2
1657	Umbellulone	−	tr
1661	*trans*-Pinocarvyl acetate	−	tr
1670	*trans*-Pinocarveol	0.4	0.4
1677	epi-Zonarene	tr	−
1682	*δ*-Terpineol	0.1	tr
1683	*trans*-Verbenol	0.2	0.2
1686	Lavandulol	−	1.2
1687	*α*-Humulene	tr	−
1697	Carvatanacetone	−	tr
1704	Myrtenyl acetate	−	0.1
1704	*γ*-Muurolene	tr	−
1706	*α*-Terpineol	2.7	0.8
1719	Borneol	tr	1.2
1722	Bicyclosesquiphellandrene	tr	−
1726	Germacrene D	1.4	−
1740	*α*-Muurolene	tr	−
1740	Valencene	−	0.2
1741	*β*-Bisabolene	−	0.1
1755	Bicyclo germacrene	0.1	−
1758	*cis*-Piperitol	−	0.1
1765	Geranyl acetate	−	0.1
1773	*δ*-Cadinene	0.7	0.1
1776	*γ*-Cadinene	0.2	tr
1783	*β*-Sesquiphellandrene	−	tr
1786	*ar*-Curcumene	0.1	−
1802	Cumin aldehyde	−	tr
1804	Myrtenol	0.3	0.5
1807	*α*-Cadinene	0.1	−
1811	*trans*-*p*-Mentha-1(7), 8-diene-2-ol	−	tr
1814	*p*-Mentha-1,5-diene-7-ol	−	tr
1823	*p*-Mentha-1(7),5-diene-2ol	0.1	0.5
1838	(*E*)-*β*-damascenone	0.1	−
1845	*trans*-Carveol	tr	tr
1849	Calamenene	tr	0.1
1864	*p*-Cymen-8-ol	tr	−
1900	*epi*-Cubebol	tr	0.3
1921	*α*-Phellandrene epoxide	tr	0.3
1941	*α*-Calacorene	tr	−
1945	1,5-Epoxy-salvial-4(14)-ene	tr	tr

RRI	Compound	A (%)	B (%)
1957	Cubebol	**0.1**	–
1969	*cis*-Jasmone	**0.2**	–
1984	γ-Calacorene	**0.1**	–
1988	2-Phenylethyl-2-methylbutyrate	**tr**	–
2008	Caryophyllene oxide	**0.1**	**0.1**
2037	Salvial-4(14)-ene-1-one	**0.1**	–
2041	Pentadecanal	**tr**	–
2056	13-Tetradecanolide	–	**0.1**
2080	Cubenol	**0.1**	**tr**
2088	1-*epi* Cubenol	**0.2**	**0.1**
2092	β-Oplopenone	**0.1**	–
2100	(*E*)-Sesquilavandulyl acetate	–	**0.1**
2109	*p*-Methoxy methylbenzoate	**tr**	–
2130	Salviadienol	**tr**	–
2131	Hexahydro farnesyl acetone	–	**tr**
2144	Spathulenol	**0.4**	**0.5**
2161	Muurola-4,10(14)-diene-1-ol	**tr**	–
2186	Eugenol	**tr**	–
2187	*T*-Cadinol	**0.4**	**0.7**
2191	Zingiberenol	**tr**	–
2209	*T*-muurolol	**0.2**	**1.8**
2219	Torreyol	**0.1**	–
2232	α-Bisabolol	**0.1**	**0.4**
2239	Carvacrol	–	**0.1**
2247	*trans*-α-Bergamotol	**0.1**	–
2255	α-Cadinol	**0.6**	**0.2**
2271	(2*E*,6*E*)-Farnesyl acetate	**tr**	**0.1**
2278	Torilenol	**tr**	–
2369	Eudesma-4(15),7-diene-1β-ol	**0.1**	–
2438	Kaur-16-ene	–	**tr**
2471	1-Heptadecanol	–	**0.1**
2500	Pentacosane	–	**tr**
2533	γ-Costol	**0.1**	–
2604	α-Costol	**0.1**	–
2622	Phytol	**tr**	**0.1**
2700	Heptacosane	**0.1**	**0.1**
2804	Benzyl salicylate	**0.1**	–
2900	Nonacosane	**0.1**	**0.1**
2931	Hexadecanoic acid	**0.8**	**0.5**
Monoterpenes		**72.1**	**51.8**
Oxygenated Monoterpenes		**16.2**	**32.9**
Sesquiterpenes		**2.9**	**0.5**
Oxygenated Sesquiterpenes		**3**	**4.3**
Others		**1.1**	**1.2**
Total		**95.3**	**90.7**

RRI: Relative Retention Indices
tr: Trace (<0.1%)
A: *Tanacetum argenteum* ssp. *argenteum* — Leaf Oil
B: *Tanacetum densum* ssp. *amani* — Leaf Oil

Table 2 Comparison of Main Essential Oil Components of *T. argenteum* and *T. densum* Subspecies in Previous Reports together with the Present Data.

Species	***T. argenteum***			***T. densum***			
Subspecies	**ssp. *canum* var. *canum***	**ssp. *flabellifolium***	**ssp. *argenteum***	**ssp. *sivasicum***		**ssp. *amani***	
Literature → / **Main Components ↓**	[3]	[2]	**A**	[9] **Fl.**	[9] **St.**	[8]	**B**
Santolinatriene	–	–	–	3.5 %	0.9 %	5.0 %	–
α-Pinene	0.4 %	29.1 %	**36.7** %	2.3 %	3.3 %	0.7 %	**9.7** %
β-Pinene	–	1.3 %	**27.5** %	2.2 %	2.3 %	0.5 %	**27.2** %
α-Thujone	11.9 %	–	–	–	–	–	–
1,8-Cineole	1.5 %	–	**9.8** %	21.1 %	28.3 %	11.5 %	**13.1** %
p-Cymene	0.1 %	0.1 %	2.1 %	0.9 %	1.5 %	6.1 %	**8.9** %
Camphor	2.5 %	14.0 %	tr	19.2 %	16.4 %	15.6 %	1.1 %
Borneol	–	0.3 %	tr	5.8 %	6.4 %	7.5 %	1.2 %
β-Patchoulene	–	–	–	–	–	17.5 %	–
β-Caryophyllene	5.1 %	3.1 %	–	–	–	0.2 %	–
Caryophyllene oxide	12.6 %	2.7 %	0.1 %	0.8 %	0.9 %	–	0.1 %
(*E*)-Sesquilavandulol	–	15.9 %	–	–	–	–	–
β-Selinene	–	–	–	–	–	5.0 %	–

Table 3 Antibacterial Activity (MIC, µg/mL) and *Vibrio fischeri* Toxicity of the Oils A and B.

Microorganism	A (µg/mL)	B (µg/mL)	+C. (µg/mL)
Staphylococcus aureus	250	>500	62.5
Methycillin resistant S. aureus	250	500	62.5
Staphylococcus epidermis	500>	500>	31.2
Bacillus cereus	125	500>	125
Bacillus subtilis	125	500>	62.5
Escherichia coli	500	500	62.5
Pseudomonas aeruginosa	250	250	31.2
Enterobacter aerogenes	500	500>	62.5
Proteus vulgaris	500>	500>	62.5
Salmonella typhimurium	500>	500>	125
Vibrio fischeri	Toxic	Toxic	N.A.

+ **C.** :Positive Control (chloramphenicol)
N.A. : Not Available

Table 4 DPPH Scavenging Evaluation of Oil A.

Concentration	A (%) *	α-tocopherol (%) *
15 mg/mL	15.6 ± 3.9	94.6 ± 0.96a
10 mg/mL	6.3 ± 4.7	94.5 ± 0.79a

* Results are given in means of three parallel experiments with S.D.

teum and *T. densum* ssp. *amani* from Turkey were investigated. α-pinene, β-pinene and 1,8-cineole rich oils were observed unlike the previous literature. Essential oil composition of *T. densum* ssp. *amani* differed from the data given in previous literature. However in order to determine chemotypes essential oil and DNA profiles of *T. densum* ssp. *amani* from various locations should be compared.

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