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Antibacterial Activity and the Variation of *Tanacetum parthenium* (L.) Schultz Bip. Essential Oils from Turkey

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Abstract: Water-distilled essential oils from herbal parts of *Tanacetum parthenium* from two different localities in Turkey were analyzed by GC and GC/MS. The essential oil of *T. parthenium* collected from Davutpaşa-İstanbul location were characterized with camphor 49%, *trans*-chrysanthenyl acetate 22.1% and camphene 9.4%. Second plant sample is collected from the remote east end of the country Şavşat-Ardahan location. The essential oil from this location was characterized with camphor 60.8% and camphene 6.8%. Unlike the former this sample contains *trans*-chrysanthenyl acetate in trace amount and *cis*- chrysanthenyl acetate in very small amount (0.6%) which is not present in the first sample. Antibacterial activity of the oils were evaluated for five Gram (+) and five Gram (-) bacteria by using a broth microdilution assay. The highest activity was observed on *Bacillus subtilis*, *Staphylococcus aureus* and methicillin-resistant *S.aureus* however when compared with positive control oils showed higher MIC values. The oil of İstanbul-sample showed highest activity on *B. subtilis* (125 μg/mL) and methicillin-resistant *S. aureus* (125 μg/mL) which is two fold concentration when compared with the positive control chloramphenicol (62.5 μg/mL). The oil of Ardahan sample showed the highest activity on *S. aureus* (125 μg/mL) which is likewise two fold concentration of the positive control chloramphenicol (62.5 μg/mL). DPPH scavenging activity was 59.3% of the oil from Davutpaşa at 15mg/mL concentration. When compared to positive control α-tocopherol (94.6%) Şavşat oil (28.2%) showed low and Davutpaşa oil showed medium DPPH scavenging activity. All of the oils showed toxicity to *Vibrio fischeri* in the TLC-bioluminescence assay.

Key words: *Tanacetum parthenium*, Asteraceae, essential oils, chemotypes, camphor, *trans*-chrysanthenyl acetate, camphene, DPPH radical scavenging, antibacterial activity, *Vibrio fischeri* toxicity

1 INTRODUCTION

T. parthenium naturally grows in the northern and southern hemisphere. This species can be found nearly in all parts of Turkey[1]. This species also known as feverfew and has many reported uses in folk medicine such as fever, migraine, menstrual disorders, stomach ache, toothache and insect bite treatments[2]. Extracts and the powder of *T. parthenium* is available in market for treatment of migraine. Also there are cases of contact dermatitis caused by this plant[3]. Reports indicate that sesquiterpene lactone content of *T. parthenium* is responsible for the observed activities[4]. Active principle is reported to be parthenolide; which is a potential agent due to its antimigraine[5], antitumor[6-8], antileishmanial[9], antiparasitic[10] and antiinflammatory[11] properties.

There are several reports on the essential oil composition of *T. parthenium*. Reports show variances in major and minor essential oil components. In most cases, camphor and chrysanthenyl acetate are the main components together with various secondary components[12-18]. According to these individual reports it seems there are two chemotypes of this plant which are camphor-camphene and camphor-chrysanthenyl acetate-camphene. Comparison of main components of *T. parthenium* previously reported together with our data is given in Table 1.

As a part of our phytochemical and biological investiga-

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Table 1 Comparison of the Previously Reported (%) Main Components of *T. parthenium* Oil and Present Data.

Compound	18) *	14) a	14) b	17)	13) c	13) d	A	B
Camphor	44.2	61.8	42.7	56.9	50.5	57.6	49.0	60.8
trans-Chrysanthenyl acetate	23.5	13.8	24.0	-	-	25.1	22.1	tr
Camphene	5.4	1.5	6.4	12.7	7.7	4.6	9.4	6.8
Germacrene- D	4.6	tr	3.0	-	9.2	0.2	-	-
p-Cymene	3.1	0.2	0.6	5.2	0.5	1.1	0.1	1.9
Bornyl acetate	0.7	1.9	1.9	4.6	1.5	1.4	2.9	3.7
(*E*)-Myrtanol	-	-	-	-	4.7	-	-	-
(*E*)-Sesquilavandulol	-	-	-	-	4.8	-	-	tr

A: Main components of essential oil from Davutpaşa sample.

B: Main components of essential oil from Şavşat sample.

*: Reference related to the main essential oil composition.

(a): Essential oil obtained from dried plant sample originated from UK.

(b): Essential oil obtained from fresh plant sample originated from Netherlands.

(c): Essential oil obtained from naturally growing wild sample originated from Iran.

(d): Essential oil obtained from cultivated sample originated from Iran.

tion of *Tanacetum* species, here we report on the composition and antibacterial, cytotoxic, radical scavenging properties of *T. parthenium* essential oil from two different locations in Turkey.

2 EXPERIMENTAL

2.1 Plant materials

Plant materials are collected during the flowering period in 17 June 2005 from Davutpaşa - İstanbul at 63 m altitude (A) and 05 July 2006 from the Şavşat - Ardahan at 2494 m altitude (B). Voucher specimens have been deposited at the Herbarium of the Faculty of Science, Istanbul University (Voucher no. ISTE 83750 and ISTE 83397 respectively), Turkey. Plant materials were identified by Dr. Kerim Alpınar.

2.2 Methods

2.2.1 Isolation of the essential oils

Flowers and stems (100 g each) of the first plant sample from Davutpaşa and second plant sample from Şavşat locations were separately subjected to hydrodistillation for 4 h using a Clevenger- type apparatus to produce the oils. Yellow colored oils with herbal-minty smell were obtained from the first and second plant samples with 0.7 % and 0.45% (v/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 2.

2.2.2.3 Identification of components

Identification of essential oil components were done by comparison of their retention times with authentic sam-

Table 2 Composition (%) of *T. parthenium* Oils from Davutpaşa and Şavşat Locations. (*Continued overleaf*)

Compound	RRI	A (%)	B (%)
Tricyclene	1014	0.4	0.3
α-Pinene	1032	0.9	1
α-Thujene	1035	0.1	0.2
α-Fenchene	1072	0.1	-
Camphene	1076	9.4	6.8
Hexanal	1093	tr	0.1
β-Pinene	1118	0.3	0.4
Sabinene	1132	0.1	0.1
Thuja-2,4(10)-diene	1135	-	0.1
Myrcene	1174	-	tr
α-Phellandrene	1176	tr	-
α-Terpinene	1188	0.1	-
Limonene	1203	0.4	0.2
β-Phellandrene	1218	tr	tr
(*Z*)-3-Hexanal	1225	-	tr
Isochrysanthenone	1234	-	0.1
2-Pentylfuran	1244	-	tr
γ-Terpinene	1255	0.2	0.1
p-Cymene	1280	1	1.9
Isoamyl isovalerate	1285	tr	-
2-Methyl butyl isovalerate	1299	tr	-
1,3,5-Trimethyl benzene	1355	-	1
1,2,3-Trimethyl benzene	1355	tr	0.7
(*Z*)-3-Hexen-1-ol	1391	-	tr
Nonanal	1400	0.1	0.1
α, *p*-Dimethylstyrene	1452	0.1	tr
Filifolene	1445	tr	0.5
Eucarvone	1465	-	0.1
Camphenilone	1474	tr	0.1
α-Amorphene	1481	-	tr
α-Copaene	1497	-	0.1
α-Campholene aldehyde	1499	tr	tr
Chrysanthenone	1522	-	3.2
Camphor	1532	49	60.8
trans-Chrysanthenyl acetate	1538	22.1	tr
Dihydro achillene	1547	0.1	tr
Linalool	1553	0.2	0.3
cis-Sabinene hydrate	1556	tr	tr
trans-*p*-Menth-2-ene-1-ol	1571	tr	0.2
cis-Chrysanthenyl acetate	1582	-	0.6
Pinocarvone	1586	0.2	-
Bornyl acetate	1591	2.9	3.7
Chrysanthenyl propionate	1599	0.3	-
Isophorone	1600	-	0.2
Terpinene-4-ol	1611	0.5	0.6
β-Caryophylene	1612	-	tr
cis-*p*-Menth-2-ene-1ol	1638	0.2	0.2
Myrtenal	1648	tr	tr
cis-Verbenol	1663	-	0.1
(*Z*)-*β*-Farnesene	1668	-	0.2
trans-Pinocarveol	1670	0.3	0.3
cis-*p*-Mentha-2,8-diene-1-ol	1678	0.1	-
trans-Verbenol	1683	0.2	0.3
Selina-4,11-diene	1688	-	0.1
trans-Piperitol	1689	tr	-
Myrtenyl acetate	1704	-	0.2
α-Terpineol	1706	0.1	0.1
Borneol	1719	0.6	tr
Verbenone	1725	0.2	0.1
β-Selinene	1742	tr	0.3
Carvone	1751	-	0.3

Table 2 Composition (%) of *T. parthenium* from Davutpaşa and Şavşat Locations.

Compound	RRI	A (%)	B (%)
cis-Piperitol	1758	0.2	tr
cis-Chrysanthenol	1764	0.2	tr
Isobornyl isovalerate	1770	0.4	-
α-Campholene alcohol	1793	0.2	0.1
p-methyl acetophenone	1797	-	0.2
Cumin aldehyde	1802	-	tr
Myrtenol	1804	-	tr
(*E,E*)-2,4-Decadienal	1827	-	tr
(*E*)-β-Damascenone	1838	-	tr
trans-Carveol	1845	0.1	0.2
Geraniol	1857	-	0.2
p-Cymene-8-ol	1864	0.1	tr
Isopiperitone	1865	-	tr
(*E*)-Geranyl acetone	1868	-	tr
cis-Carveol	1882	0.1	0.6
Benzyl-2methylbutyrate	1880	0.2	-
cis-p-Mentha-1(7),8-diene-2-ol	1896	0.1	0.4
Benzyl isovalerate	1902	tr	-
trans-Jasmone	1948	-	tr
(*E*)-β-Ionone	1958	-	tr
Bornyl angelate	2118	0.1	-
Caryophyllene oxide	2008	0.2	1.1
Salvial-4(14)-en-1-one	2037	tr	tr
Humulene epoxide II	2071	-	tr
Caryophylla 2(12), 6(13)diene-5one	2074	tr	0.2
Cumin alcohol	2113	tr	0.1
Hexahydro farnesyl acetone	2153	0.1	0.1
Muurola-4,10(14)-dien-1-ol	2161	-	tr
(*E*)-Sesquilavandulol	2183	-	tr
Eugenol	2186	0.2	-
Copaborneol	2210	0.1	tr
Nonanoic acid	2192	tr	0.2
Thymol	2198	0.1	-
Carvacrol	2239	tr	0.2
β-Eudesmol	2257	0.1	-
Intermedol	2264	tr	0.5
Selin-11ene-4α-ol	2273	0.1	0.3
Decanoic acid	2298	-	0.7
Caryophylladienol I	2316	tr	0.3
Caryophylladienol II	2324	0.1	0.4
Tricosane	2300	0.1	-
Caryophyllenol I	2389	-	0.2
Caryophyllenol II	2392	0.1	0.3
Pentacosane	2500	0.1	0.4
1-Octadecanol	2607	0.2	-
Heptacosane	2700	tr	tr
Tetradecanoic acid	2670	0.4	0.2
Nonacosane	2900	0.1	0.1
Hexadecanoic acid	2931	0.3	0.7
Monoterpenes		**13.1**	**11.1**
Oxygenated Monoterpenes		**78.5**	**73.6**
Sesquiterpenes		**0**	**0.7**
Oxygenated Sesquiterpenes		**0.8**	**3.4**
Others		**1.8**	**4.6**
Total		**94.2**	**93.4**

RRI: Relative Retention Indices

tr: Trace (<0.1%)

* Correct isomer not identified

A: Davutpaşa sample - Herb Oil

B: Şavşat sample - Herb Oil

Table 3 Antibacterial (MIC) and Cytotoxic Activity of *T. parthenium* Oils from Davutpaşa (A) and Şavşat (B) Locations.

Microorganism	A (μg/mL)	B (μg/mL)	+ C. (μg/mL)
Staphylococcus aureus	250	125	62.5
Meticillin resistant *S.aureus*	125	500	62.5
Staphylococcus epidermis	500	500>	31.2
Bacillus cereus	500	500>	125
Bacillus subtilis	125	500>	62.5
Escherichia coli	500	500>	62.5
Pseudomonas aeruginosa	500	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

ples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library)[20,21] 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[22-24] was used for identification.

2.2.3 Antibacterial activity assay

Five Gram-positive bacteria (*S. aureus* ATCC 6538, *S. epidermis* ATCC 12228, *B. cereus* NRRL B-3711, *B. 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[25]. Stock solutions of the oils (2 mg/mL) and standart antibacterial compound chloramphenicol (2 mg/mL) were prepared. Liquid medium was diluted by adding 25% DMSO or CH_3OH. Serial dilution was done on 96-well microlitre plates. Bacteria were standardized according to McFarland No:0.5 after incubation 24 h at 37℃ on 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 *V. fischeri* toxicity in Table 3.

2.2.4 *V. fischeri* cytotoxicity assay

5μL 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 *V. 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[26]. The results of *V. fischeri* toxicity activity test are given together with antibacterial activity results in Table 3.

2.2.5 DPPH scavenging property assay

Antioxidant activity of the oils were determined with DPPH radical protocol[27]. A modified protocol for HPTLC-DPPH[28] was used. Stock solutions of the oils from both samples (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 one hour 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 applicator 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

Table 4 DPPH Scavenging Activity of the Essential Oils*.

Conc.	A (%)	B (%)	α-tocopherol (%)
15 mg/mL	59.3 ± 2.58	17.3 ± 5.05	94.6 ± 0.96
10 mg/mL	28.2 ± 5.36	5.2 ± 0.39	94.5 ± 0.79

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

property was calculated according to % DPPH Scav. Prop. = [(AControl-ASample)/ AControl]×100 formula. The results of antioxidant property activity tests are given in Table 4.

3 RESULTS

Essential oil compositions of Davutpaşa and Şavşat samples are given in Table 2. In Davutpaşa sample, seventy six compounds were detected representing 94.2% (A) of the oil. Ninety-two compounds were identified in the oil of the sample from Şavşat representing 93.4% (B) of the oil. Oil obtained from Davutpaşa sample was rich in camphor 49%, *trans*-chrysanthenyl acetate 22.1% and camphene 9.4%. Previously same main components were reported from Belgium[18], Netherland[14] and Iran[13]. Essential oils of the Şavşat sample was rich in camphor and camphene unlike Davutpaşa sample which contained *trans*-chrysanthenyl acetate in high quantities together with these compounds. *trans*-Chrysanthenyl acetate also occur in Şavşat sample with trace amounts (<0.1%). A *T. parthenium* oil reported in the previous literature from Sivas-Turkey which is similar to Şavşat oil. However this oil contained higher amount of 12.7 % camphene, 5.2% *p*-cymene and 4.6% bornyl acetate[17].

Davutpaşa oil showed mild activity on meticillin resistant *S. aureus* (125 μg/mL) and *B. subtilis* (125 μg/mL) with two fold concentration when compared to the positive control chloramphenicol (62.5 μg/mL). Şavşat oil showed mild activity to *S. aureus* (125 μg/mL). Şavşat oil did not show any significant activity towards meticillin resistant *S. aureus, B. subtilis* nor Davutpaşa oil to *S. aureus*. Both oils did not have any significant activity against the other organisms. Both oils showed toxicity to *V. fischeri* with HPTLC-*V. 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.

DPPH radical scavenging activity was observed on the samples with 15 mg/mL concentration. Essential oil from Davutpaşa sample showed higher scavenging activity (59.3%) than the oil from Şavşat sample (17.3%). When compared to positive control α-tocopherol (94.6%) none of the oils showed significantly high DPPH scavenging activity.

4 DISCUSSION

Differences can be clearly seen on the main components of *T. parthenium* essential oil from the previous literature and the present work in Table 1. However these differences seen in Table 1 could be related to different collection times, climatic and soil conditions, ecological factors, methods and instruments employed in analysis or different genotypes. These differences arising from the occurence/absence of compounds *trans*-chrysanthenyl acetate and sesquiterpenes such as germacrene D, (*E*)-sesquilavandulol suggests that differences could be related to absence/occurence or regulation of specific enzymes that are responsible for the biosynthesis of these compounds. In both Davutpaşa and Şavşat oils caryophyllene, eudesmane and cadinene types are dominant in sesquiterpenes. However germacrene type was not present. Sesquiterpenes of eudesmane and cadinene types are known to be formed by carbocationic cyclization reactions from germacryl cation. However caryophyllene type compounds are formed by carbocationic cyclization reactions from humulyl cation and have different biosynthetic origin[19]. DPPH-Scavenging assay and antibacterial activity assays also confirmed the variation of the oils from Davutpaşa and Şavşat. In order to determine the possible reason of this variations DNA profiling studies are required.

5 CONCLUSIONS

Essential oil compositions of *T. parthenium* from two different locations in Turkey were investigated. Camphor and *trans*-chyrysanthenyl acetate-camphor rich oils were observed which is similar to the previous literature. However secondary components accompanying main components like germacrene-D and (*E*)-sesquilavandulol were not present unlike previous literature data. DNA profiling studies are required for determining the chemotypes of *T. parthenium*, which could explain the variations observed in the oil compositions. None of the oils showed significant activity against tested microorganisms. Likewise DPPH scavenging activities of the oils were not significant. However both oils showed toxicity to *V. fischeri*.

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