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ESSENTIAL OIL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ENDEMIC *TANACETUM DENSUM* SUBSP. *AMANI* HEYWOOD FROM TURKEY

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Abstract

Essential oils from the aerial parts of *Tanacetum densum* subsp. *amani* (Astareceae) collected from Kahramanmaraş city, were obtained by hydrodistillation method and chemical composition of the oil was analyzed by GC-MS/ FID. In antimicrobial assays, chloroform and methanol extracts of the plant were tested against seven bacteria namely, *B.megaterium* DSM 32, *E.aerogenes* ATCC 27859, *E.coli* ATCC 25922, *P.aeruginosa* 9027, *S.aureus* ATCC 25923, *B.subtilis* IMG 22, *K.pneumoniae* FMC 5, and three yeasts such as *S.cereviciae* WET 136, *Y. lipolytica* MB3, *C.albicans* ATCC 1023, and concentration of miniumum inhibiton were determined. As a result, 1,8 cineol (17.64 - 28.26%), α–pinene (3.54 - 15.75%), 2-hexene, 5 methyl (6.72 - 8.67%), camphor (9.15 - 6.68%), borneol (9.17 - 6.18%) and piperitenone (5.43 - 6.50%) were found as main components with MS and FID respectively. MIC activity of extracts inhibited some microorganisms growing in 25-75 µg/ µl concentrations. The chloroform extracts were found to be more effective than methanol extracts and exhibited significant antimicrobial activity but did not show any activity against to *Candida albicans.*

Introduction

Tanacetum densum subsp. *amani* (Astareceae) is an endemic plant in Turkey. Astereceae has some members which have traditionally been used in balsams, cosmetics, dyes, medicines and preservatives as herbal remedy (Akpulat *et al*. 2005). The genus *Tanacetum* contains, totaling over 200 species and distributed over West Asia and Europe (Kumar and Tyagi 2013). Recent studies have also shown that the essential oils or extract of *Tanacetum* exhibits anti-inflammatory (Mordujovich-Buschiazzo *et al.* 1996), antibactericidal, antifungicidal (Neszmelyi *et al.* 1992) antifeedant activity (Susurluk *et al.* 2007) and migraine therapy effect against to headache because of its high melatonin content (Dıraz 2015; Murch *et.al* 1997). Terpenes in the essential oil are thought to associate with the biological activity of *Tanecetum* (Akpulat *et al*. 2005). The composition of tansy oils varies markedly and several chemotypes from different geographical origins have already been classified and some chemotypes such as; α-thujone, β-thujone, camphor, 1,8-cineole, borneol, chrysanthenone or dhiydrocarvone, artemisia alcohol, camphenol, davadone, lyratol, lyratyl acetate, artemisia ketone, chrysanthenyl acetate were detected (Keskitalo *et al*. 2001). Lawrence described 23 chemotypes in tansy oils and same author reported commercial tansy oils are mostly thujone types (Lawrence 2000). The composition of the different Turkish *Tanecetum* taxa have been reported by some authors (Baser *et al*. 2001; Goren *et al*. 2001; Polatoglu *et al*. 2006; Kılıc 2014) *T. densum* subsp. *amani* Heywood is an endemic plant and widespread in Turkey. Some publications on sesquiterpenes of this taxon have been found (Cogoa *et al*. 2012, Polatoglu *et al*. 2011). Phenolic contents and antioxidative properties of *T. densum* subsp. *amani* were studied by Tepe and Sokmen (2007). Some works on the essential oil

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composition of the plant from different cities in Turkey are available in the literature (Kızıl *et al*. 2009, Bagcı 2009, Polatoglu *et al*. 2009). Presence of antimicrobial activity of the essential oils of *T. densum* subsp. *amani* was reported by Polatoglu *et al*. (2012) that there was no significant antimicrobial effect of the *T. densum* subsp. *amani* on the bacteria. From the literature survey no report on the differences of antimicrobial activity of chloroform and methanol extracts about this endemic plant was as certained. Therefore, in the present study the essential oil composition were analysed to compare with different localities in the reference. Antimicrobial and MIC activities of different extracts (chloroform and methanol) isolated from the aerial parts of *T. densum* subsp. *amani* were investigated.

Materials and Methods

Flowering plant material of *T. densum* subsp. *amani* was collected from a wild population of Kahramanmaras provinces Ahirdag Mountain, Yedikuyu and identified by the taxonomist Dr. Ahmet İlçim using Flora of Turkey and East Aegean Islands (Grıerson 1975). A voucher specimen has been deposited in the herbarium of the Faculty of Science, KSU in Kahramanmaras, Turkey (Collector Number: A. Ilcim 1477 KSUH).

The air dried flowering parts of the plants were distilled for 3 hrs using a Clevenger type apparatus. The sample oils analysed by using GC-MS/FID. Analysis was conducted in the Plant Physiology Laboratory in Biology Dept. of KSU. Qualification of the oil was analyzed on an Agilent 5975C Mass Spectrometer coupled with Agilent GC-6890II series. The GC was equipped with HP-88 capillary column (100 m × 250 μm × 0.20 μm film thickness) and flow rate of carrier gas was 1.0 ml/min. Oven temperature of GC was programmed as follows: 70°C (1 min), 230°C at 10°C/min and then kept at 230°C at 20 min. The injection part temperature was 250°C. The mass spectrometer was operating in EI mode at 70 eV. Split ratio was 20 : 1. Mass range 35 - 400 m/z; scan speed (amu/s): 1000. 10 μl of the oil was shaked in 0.5 ml diethyl ether and 1 μl of the mixture was injected into the column.

The antimicrobial activities of chloroform and methanol extracts from the *T. densum* subsp. *amani* were tested against seven bacteria (*Bacillus megaterium* DSM 32, *Bacillus subtilis* IMG 22, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumonia* FMC 5, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* 9027 and *Enterobactera erogenes* ATCC 27859) and three yeasts (*Candida albicans* ATCC 1023, *Saccharomyces cereviciae* WET 136 and *Yarrowia lipolytica* MB 3). The microbial strains used in this study were obtained from the Microbiology Laboratory Culture Collection, Department of Biology, KSU, Turkey.

The leaves of *T. densum* subsp. *amani* were crushed (1 g) and extracted with chloroform and methanol solvents (1 ml) for 24 hrs. The disk diffusion method was used for antimicrobial activities of *T. densum* subsp. *amani* extracts. These mentioned bacteria were incubated at 37 ± 0.1°C for 24 hrs by injection into nutrient broth (Difco), and the yeasts were incubated in sabouraud dextrose broth (Difco) at 25 ± 0.1°C for 24 hrs. Mueller Hinton agar (MHA) (Oxoid) and Sabouraud dextrose agar (SDA) were sterilized in a flask and cooled to 45 - 50°C and were distributed homogenously to sterilized Petri dishes having a diameter of 9 cm (25 ml) after injecting cultures (0.1 ml) of bacteria and yeast (Mc Farland OD : 0.5, 1.5×10^8 bacteria/ml and 1.5×10^6 yeast/ml) (NCLS., 1999, NCLS. 2007). Subsequently, *T. densum* subsp. *amani* extracts 100 micro liters (10 mg) in chloroform and methanol solutions were pipetted into the sterile blank paper disks (at 11 mm diameter). Afterward, the plates combined with the disks were kept at 4°C for 2 hrs, the plates injected with yeast were incubated at 25 ± 0.1°C for 24 hrs, and ones injected with bacteria were incubated at 37 ± 0.1°C for 24 hrs. After 24 hrs, inhibition zones which appeared around the disks were measured and recorded in mm (Bradshaw 1992).

A broth micro dilution broth susceptibility assay was used, as recommended by NCCLS (NCCLS 2006), for the determination of the MIC of the *T. densum* subsp. *amani* chloroform and methanol extracts. All tests were performed in Mueller Hinton broth (MHB) supplemented with Tween 80 detergent (final concentration of 0.5%, v/v), with the exception of the yeasts (sabouraud dextrose broth (SDB) + Tween 80). Bacterial strains were cultured overnight at 37°C in MHB, and the yeasts were cultured overnight at 25°C in SDB. Geometric dilutions of the chloroform and methanol extracts including one growth control (MHB + Tween 80) prepared with the 25 to 100 µg/µl. Test tubes were incubated under normal atmospheric conditions at 37°C, 24 hrs for bacteria and at 25°C, 48 hrs for the yeasts. The microbial growth was determined by turbidimetric methods at 550 nm.

Results and Discussion

The results obtained on essential oil composition of *Tanacetum densum* subsp. *amani* and GC-MS chromatogram are presented in Table 1 and Fig. 1, respectively. According to FID and MS results, 32 components were identified, which accounted of the total oil for 99.08% FID and 89.17% with MS detector. The components percentage showed high differences with MS and FID detectors. Butanoic acid, methylbuthyl-2-isovalerate, carvone, spathulenol were analysed with only FID detector while sabinene, limonene, Θ-terpinen, cyclododecanone were analysed with only MS detector.

Table 1. Essential oil composition of the *T. densum* subsp. *amani*.

Compound	RT	A	B	Compound	RT	A	B
α -pinene	11.22	15.75	3.54	Trans pinocarveol	20.20	1.62	3.07
Camphene	11.78	1.69	1.88	Camphor	20.45	6.68	9.15
β-pinene	12.17	2.62	0.64	Borneol	21.03	6.18	9.17
Sabinene	12.32	0.61	-	Myrtenal	22.57	0.79	1.45
Limonene	12.86	0.90	-	Myrtenol	21.78	0.69	1.17
Θ-terpinen	13.40	0.37	-	Trans-carveol	22.00	0.68	1.26
1,8 cineol	14.00	28.26	17.64	Carvone	21.43	-	0.36
Ortho-cymene	14.38	2.11	3.11	Cyclododecanone	22.83	0.69	2.10
Butanoic acid	14.45	-	1.02	Shiso furan	23.12	0.49	1.05
2-methylbutyl isovalerate	14.56	-	0.43	Verberone	23.76	0.34	0.82
Isoamyl-2-methyl butyrate	14.96	0.35	0.35	Oxacyclotetradecan-2-one	24.71	1.20	2.67
Octanal	15.68	0.10	0.6	Spathulenol	24.83	-	1.93
2-hexene, 5 methyl	16.12	8.67	6.72	Muurola-4(14),5 diene	24.97	0.29	1.07
α-thujone	17.73	0.28	0.32	Piperitenone	25.69	6.50	5.43
Ipsidenol	18.76	0.79	0.71	Carvacrol	26.25	1.23	0.43
α-campholenal	19.10	2.19	4.39	Isoeugenol-Z	26.92	0.33	0.30
Bornyl acetate	19.47	1.45	5.14	Intermedeol	27.51	3.24	0.38
z-citral	19.96	0.39	0.87	Cyclocolorenone	32.18	1.60	-

RT: Retention time A: % FID results. B: % MS results.

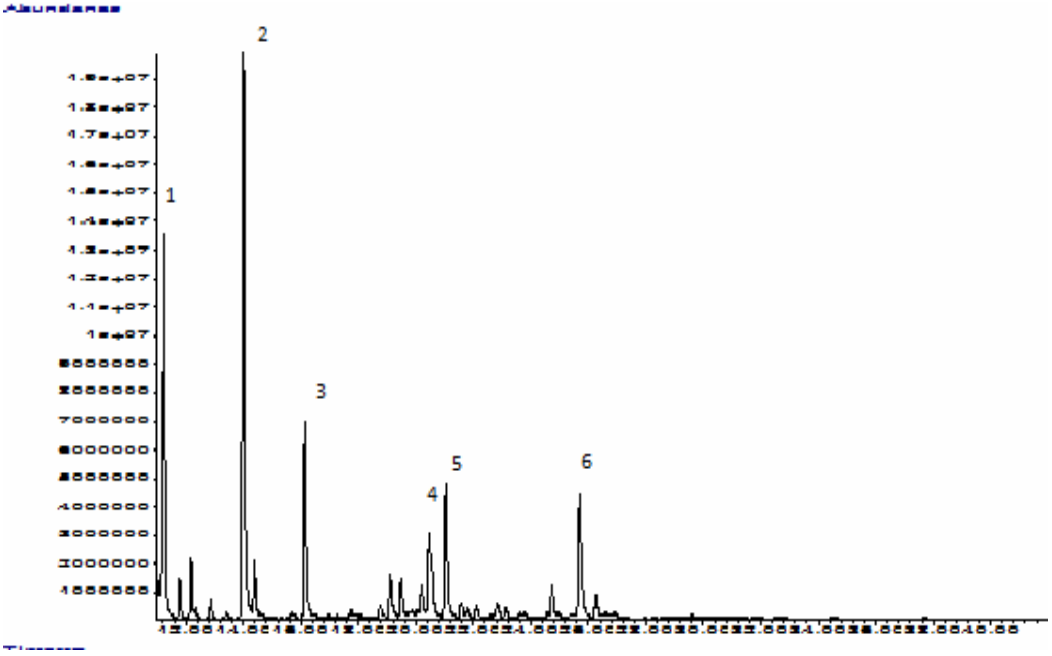

Fig. 1. GC-MS chromatogram of *T. densum* subsp. *amani* essential oils.

Peaks were identified as retention times and given, respectively. 1: α -pinene, 2: 1,8 cineol, 3: 2-hexene, 5 methyl, 4: camphor, 5: borneol, 6: piperitenone

As reported subspecies of *T. densum* showed important differences in their chemical composition. These quantitative differences about main compounds reflect the different extrinsic conditions and collection dates. Kokkini *et al*. (1995) had reported that the season of collection strongly affects the oil yield of the plants. Meanwhile, Circella *et al*. (1993) reported that the climatic factors can influence the differentiation and the secreting activity of the glands located in the epidermis and even the extraction method may cause variation in the composition of volatile oil. In particular, elevated temperatures during distillation can cause chemical changes in the resultant oil. These differences may be considered as an indication of chemo types existing within the subspecies. Also, when the data presented in this paper compared with other reports for same subspecies of *Tanacetum* show that there are differences in the amount of same compounds.

Antimicrobial activity and MIC results of *T. densum* subsp. *amani* chloroform and methanol extracts are presented in Tables 2 and 3. As seen in the Table 2, extracts of *T. densum* subsp. *amani* inhibited the test microorganisms specially bacteria (11 - 19 mm inhibition zone) in 100 µg/µl. The chloroform extracts were found to be more effective than methanol extracts. *T. densum* subsp. *amani* extracts did not show any activity against to *C. albicans.* Chloroform extracts of *T. densum* subsp. *amani* are highly active on *K. pneumoniae* and *P. aeruginosa* and growth large inhibition zone as 18 - 19 mm. Data presented in the Table 2 were analysed via SPSS program followed by univariate analyses (ANOVA) using Waller-Duncan test. All trials were replicated three times.

Table 2. Antimicrobial activity of *T. densum* subsp. *amani* extracts.

Microorganisms	Chloroform extracts*	Methanol extracts*
B. megaterium DSM 32	17 ± 2.82^{ab}	12 ± 0.70^{a}
K. pneumoniae FMC 5	19 ± 1.40^{a}	12 ± 0.70^{a}
E. coli ATCC 25922	16 ± 1.41^{abc}	12 ± 1.41^{a}
P. aeruginosa 9027	18 ± 2.12^{ab}	11 ± 1.41^{a}
S. aureus ATCC 25923	13 ± 2.12^{bc}	0^{b}
B. subtilis IMG 22	17 ± 1.41^{abc}	11 ± 0.70^{a}
E. aerogenes ATCC 27859	14 ± 0.70^{abc}	11 ± 1.40^{a}
C. albicans ATCC 1023	0^{d}	0^{b}
Y. lipolytica MB3	12 ± 1.41^{bc}	11 ± 0.00^{a}
S. cereviciae WET 136	0^{d}	0^{b}
Mean value±standard error	12.692 ± 4.41	8.911 ± 1.43
Control	0	0

Standard devisions were given as ± with mean value of repetitions. Mean values were analysed with comparing control groups. Differences on microbial strains and solvents were found important as statistically ($*p < 0.01$).

Table 3. MIC activity of *T. densum* subsp. *amani* extracts.

Microorganisms	MIC (µg/µl)	Microorganisms	MIC (µg/µl)
B. megaterium DSM 32	25	*E. aerogenes* ATCC 27859	50
	75		75
K. pneumoniae FMC 5	25	*C. albicans* ATCC 1023	>100
	75		>100
E. coli ATCC 25922	50	*Y. lipolytica* MB3	75
	75		75
P. aeruginosa 9027	25	*S. cereviciae* WET 136	>100
	75		>100
S. aureus ATCC 25923	50	Control	>100
	>10		>100
B. subtilis IMG 22	25		
	75		

In the results of MIC, chloroform and methanol extracts inhibited some microorganisms growing in 25 - 75 µg/µl concentrations. Especially, It was seen that MIC value of chloroform extracts were lower than methanol extracts (Table 3). Inhibition growth of fungal species *C. albicans* could not be determined >100 µg/µl concentration. Antifungal effect of solvents extracts on *C. albicans* were not determined.

Polatoglu *et al.* (2012) investigated the antimicrobial activity of *T. densum* ssp. *eginense* with same method on the same microorganisms and oils did not show any significant activity to the tested microorganisms, as well. The differences may be considered from extraction method which may cause variation in the composition of volatile oil (Keskitalo *et al*. 2001). The present findings about antifungal effects are in agreement with the results of Polatoglu *et al*. (2006) who found antifungal activity in two subspecies of *T. densum*. In previous report Stojkovic *et al*. (2011), 1,8 cineol obtained from *Vitexagnus-castus* oil showed high antimicrobial activity and 1,8 cineol completely inhibited *Aspergillus* root development in apple fruits. Previous reports on Turkish *Tanacetum* species showed high levels of antimicrobial activity which include the 1,8 cineol as major component (Salamcı *et al*. 2007), reported that antifungal assays with oil of *T. aucheranum* and *T. chiliophyllum* var. *chiliophyllum,* showed that the oils completely inhibit the growth of 30 phytopathogenic fungi and considerable antibacterial activity over a wide spectrum against 33 bacterial strains as positive standards. In a study of Tabanca *et al*. (2007), antimicrobial activity of the *T. argenteum* subsp. *flabellifolium* essential oil showed good growth inhibitory effects against *P. aeruginosa, E. aerogenes,* and *C. albicans* with MIC values of 125 g/ml. The *T. argenteum* subsp. *flabellifolium* oil demonstrated weak to moderate growth inhibition against the pathogenic bacteria *E. coli, S. aureus, P. vulgaris* and *S. typhimurium*. The present results also are supported by Tabanca *et al*. (2007) where *T. densum* extracts were highly active on *P. aeruginosa* and *C. albicans* strains, and showed antibacterial activity on *E. coli* and *S. aureus* strains. On the contrary Goren *et al*. (1996), reported that most of sesquiterpene lactones from *T. praeteritum* subsp. *praeteritum* were not effective in the antimicrobial test. Keles *et al*. (2001), reported that ethanol extracts of two *Tanacetum* species; *T. parthenium* and *T. vulgare* collected from Ilgaz/Kastamonu city from Turkey had moderate antibacterial effect against 7 bacteria that they had inhibition zone (between 8 to 14) against *Klebsiella pneumoniae, Staphylococcus aereus* and *Salmonella gallinarum* and these two species had different MIC values.

Additionally Polatoglu *et al*. (2006), reported that none of the two subspecies oils showed cytotoxic or antileishmanial activities. Also the flower oils of the two species showed significant phytotoxic activity on *Lemna minor* growth. Tepe and Sokmen (2007) reported that methanol extract of *T. densum* subsp. *amani* had high antioxidant activity.

From the present study it may be concluded that main components and their ratio of the essential oils of *T. densum* subsp. *amani* are highly effected from environment, also chloroform extract of the plant showed higher antimicrobial effect. The plant extract may be useful as a natural fungicide and bacteriostatic.

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