International Journal of Pharmacognosy and Pharmaceutical Research
www.pharmacognosyjournals.com
Online ISSN: 2664-7176; Print ISSN: 2664-7168
Received: 01-04-2019; Accepted: 03-05-2019; Published: 10-05-2019
Volume 1; Issue 1; 2019; Page No. 18-22

Essential oil and fatty acid composition leaves of some aromatic plant taxa from turkey

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Abstract
In this research essential oil and leaf fatty acid compositions of *Tanacetum densum* subsp. *eginense, Inula germanica*, *Grammosciadium macrodon* and *Seseli peucedanoides* were analyzed by GC/GC-MS. Borneol (20.7%), 1-8 cineole (13.4%) and Camphor (12.4%) were detected the main compounds of *Tanacetum densum* subsp. *eginense*; □□-caryophyllene (20.4%), Germacrene D (11.4%) and Borneol (11.3%) were detected the major constituents of *Inula germanica*; Caryophyllene oxide (12.5%), Camphor (11.8%) and□□-3-carene (10.4%) were determined the main compounds of *Grammosciadium macrodon*; Limonene (21.2%), □-Pinene (12.6%) and □-Phellandere (11.1%) were found to be the main compounds of *Seseli peucedanoides.* The major fatty acid composition of studied samples were found to be Palmitic (21.84-47.48%), Steraic (11.96-31.09%), Petroselinic (2.91-15.74%), Linoleic (6.46-33.77%) and Linolenic acid (5.24-35.25%); while other fatty acids were found in small proportions.

Keywords: essential oil, fatty acid, asteraceae, apiaceae

Introduction

The genus *Tanacetum* L. (Emend. Briq.) which is an important member of the Compositae family, this genus contains in Turkey many endemic taxa and endemism rate 40%; *Tanacetum densum* (Lab.) Schultz Bip. subsp. *eginense* Heywood is also an endemic taxa in Flora of Turkey [1]. *Tanacetum* is the third largest genus of Asteraceae and represented by about 160 species worldwide [2]; it has mostly perennial, rarely annual species and the species of *Tanacetum* taxa are generally scattered in Europe, Asia, North Africa, and North America. Some species of the Tanacetum are cultivated and the habits of the taxa are vary from herbs to subshrubs [3]. More than forty-six species are found in Turkey belonging to the *Tanacetum* [4]. *Tanacetum* taxa are rich in essential oils and they have many biological activities and uses as alternative remedies for the therapy of many illness [5]. Some *Tanacetum* taxa have been used as ornamental plant, used in salads, omelets, cakes, dyes, medicines, cosmetics and in ethnobotany [6]. Within *Tanacetum* taxa, the essential oil composition differs significantly, depending on the geographical origins, ecological conditions, different regions of the plant and developmental stages; the review showed that commercial oils of tansy are mostly of the thujone type. Thujone has medicinal properties, but at high concentrations it exhibits toxicity, characterized especially by neurological effects [7]. The major constituents of the essential oils of Tanacetum taxa were: thujone derivates, camphor, trans-chrysanthenyl acetate, sabinene, borneol, myrtenol and 1,8- cineole, with the quantities and proportions of each varying seasonally and from plant to plant [8-10].

In Turkey *Inula* L. (Compositae) genus is comprises about 32 taxa [11]; most of *Inula* taxa have benefical effects for human healthy [12]. *Inula germanica* L. is a rhizomatous perennial herb; stem erect 35-75 cm, densely leafy; leaves ovate or oblanceolate; capitula campanulate-radiate; involucre 0,75-1 cm broad [1]. The characteristic compounds of the Inula taxa are sesquiterpenes and monoterpenes [13]. Further phytochemical studies of Inula sp. are needed to support classification efforts, which nowadays are based mainly on morphological traits. The essential oil of Inula is known as the most effective oil for loosening, deep congestion, coughs, colds, sinusitis larygitis and bronchitis; moreover it suppots lymphatic circulation as well as the immune system, it also reduces acneic skin inflammation [12]. Besides several Inula species have been used in various treatments: for calculus, for eye, as diuretic, position and sudorific. The species of Inula genus are lack detailed phytochemical investigation. Essential oils are valuable natural products used as raw materials in many fields such as perfumes, cosmetics, aromatherapy, spices and nutrition [14]. Members of the genus Grammosciadium DC. are among the most important aromatic plants and the commercial potential of this genus as a source of essential oils has already been reported [15].

Grammosciadium macrodon Boiss. and *Seseli peucedanoides* (Bieb.) Koso-Pol. are in the Apiaceae family; this family is rich in secondary metabolites and have many economic valuable plants [16]. Essential oil of some *Grammosciadium* DC. and *Seseli* L. taxa have antibacterial, antioxidant, free radical scavenging activity and usefull effects for human health [17-19]. Some species of *Grammosciadium* are known to be used in various food preparations with carminative and relief stomachache properties; fresh or dried herbal parts of this plant are used as a local vegetable and flavoring in soups and foods in the collection site. As far as our literature survey could ascertain, there have been very lack attempts to investigate the essential oil composition Grammosciadium species grown in Turkey. Some Seseli species have been used in folk medicine since ancient time against human inflammation, swelling, rheumatism, pain, common cold as well as exhibited antihelmintic, and carminative activity [17].

In this research, essential oil and fatty acid composition leaves of *Tanacetum densum* subsp. *eginense*, *Inula germanica*, *Grammosciadium macrodon* and *Seseli peucedanoides* were detected. This is the first report on the leaf fatty acid composition of *Tanacetum densum* subsp. *eginense*, *Inula germanica*, *Grammosciadium macrodon* and *Seseli peucedanoides* from Turkey.

Materıals and methods

Plant Materials

Tanacetum densum subsp. *eginense* and *Grammosciadium macrodon* were collected north of Genç district (Bingöl), vicinity of Haziran village, steppe and rocky areas, in June 2018, (Kilic 3621, 3628). *Seseli peucedanoides* was collected Solhan (Bingöl), Hazarşah village, Aksakal Gol position, volcanic stony areas of river, May 2017, (Kilic 2842). *Inula germanica* was collected from Kemaliye (Erzincan), Kadik district, steppe, rocky areas, June 2017, (Kilic 2932). Plant samples were identified with Flora of Turkey and East Aegean Islands [1]. Voucher specimens were deposited in the Department of Park and Garden Plants of Technical Vocational College/Bingol University.

Isolation of the essential oil

Essentiai oils were obtained with hydrodistillation method, using a Clevenger-type apparatus for 2.5 hours.

Gas Chromatography/Mass Spectrometry (GC-MS) Analysis

5 g of the plant samples were homogenized in 10 mL of hexane/isopropanol at 10.000 rpm for 30 second and centrifuged at 5000 rpm for 10 min [20]. The upper part was taken and put into the test tubes by filtration. Fatty acids need to be derivatized in order to be able to look at GC. Derivatization with methyl esters is often preferred. For this purpose Christie method [21] was used. 5 mL of 2% methanolic sulfuric acid was added and vortexed. This mixture was kept at 50 ^{0}C for 15 hours of methylation. After 15 hours, the tubes were removed, cooled to room temperature, and vortexed with the addition of 5 mL of 5% NaCl. The fatty acid methyl esters formed in the tubes were extracted with 5 mL of hexane and the hexane phase was removed from the top by a pastry pipette and treated with 5 mL of 2% $KHCO_3$ and waited for 1-2 hours to separate the phases. The solvent of the mixture containing the methyl esters was then evaporated at 45°C under nitrogen and the fatty acids below the test tubes were dissolved in 1 mL of hexane and analyzed by GC-MS using amber GC vials. The essential oils were analyzed using HP 6890 GC equipped with and FID detector was used. The column and analysis conditions were the same as in GC-MS. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors. The essential oil compounds were identified using the Wiley and Nist mass spectral library and the identified compounds of the essential oils are listed in Table 1. An Agilent brand 7890A / 5970 C GC-MS instrument and a SGE Analytical BP x 90 100m x 0.25 mm x 0.25 um column were used for fatty acid analysis. The temperature program was gradually heated from 120 ^{0}C to 250 ^{0}C and the total time was set to 45 min. The temperature program is like this; 120 ^{0}C is heated up to 250 ^{0}C at 5 ^{0}C / min and is expected at this temperature for 19 min and the total time is 45 min. The autosamplers washed themselves in hexane 5 times before shrinking and after giving the collar. Injection volume was 1 uL and split ratio was 10: 1, solvent delay time was 12 minutes, carrier gas was He, and H_2 flow was 35 mL / min, flow rate was 350 mL/min, N_2 was 20.227 mL/min is automatically set by the program. The identified fatty acid compounds of studied taxa are listed in Table 2.

Results and dıscussıon

In this study leaf essential oil and fatty acid compositions of *T. densum* subsp. *eginense, I. germanica*, *G. macrodon* and *S. peucedanoides* were analyzed by GC and GC-MS. The major fatty acid compounds of studied taxa as were: palmitic (21.89%), stearic (11.96%), linoleic (14.12%), linolenic acid (35.25%) in *T. densum* subsp. *eginense*; palmitic (31.72%), linoleic (20.47%), stearic (18.18%), linolenic acid (13.93%) in *I. germanica*; palmitic (30.62%), stearic (19.88%), petroselinic (15.74%), linoleic acid (33.77%) in *G. macrodon*; palmitic (47.48%), stearic (31.09%), linoleic acid (6.46%) in *Seseli peucedanoides* (Table 2). Borneol (20.7%), 1-8 cineole (13.4%) and camphor (12.4%) were detected main essential oil compounds of *T. densum* subsp. *eginense*; □□-caryophyllene (20.4%), germacrene D (11.4%) and borneol (11.3%) were detected major constituents of *Inula germanica*; caryophylleneoxide (12.5%), camphor (11.8%) and□□-3-carene (10.4%) were determined the main compounds of *G. macrodon*; limonene (21.2%), □-pinene (12.6%) and □-phellandere (11.1%) were found to be the main compounds of *Seseli peucedanoides* (Table 1).

In a study essential oil of *Seseli peucedanoides* was analyzed; as a result forty-six compounds were detected representing 96.3% of the oil, with α-pinene (69.4%), β-pinene (4.9%) and limonene (4.6%) found to be main compounds [22]. Similarly α-pinene (12.6%), limonene (21.2%) and β-pinene (4.8%) were detected the main compounds of *Seseli peucedanoides* (Table 1). In another study, the essential oils of flowers, leaves, stems and roots of *Seseli rigidum*, wild growing in Bulgaria were analyzed and □-phellandrene was the major component in the flower (47.5%) and stem (63.1%) oils; sabinene (39.8%) was the main compound in the leaf oil and also a dominant one in the stem (6.5%) and flower (19.8%) oils. In the literature sabinene and □-phellandrene have been reported to dominate in oils from *Seseli buchtormense* [23]. It is noteworthy that in our study limonene (21.2%), □-pinene (12.6%) and □-phellandere (11.1%) sabinene (8.2%) were found to be the main compounds of *Seseli peucedanoides*; unlike this work in our study limonene was found to be major constituents (Table 1). In another study limonene (28.4%), β-pinene (16.1%), β-selinene (15.9%), δ-3-carene (11.2%), α-farnesene (6.0%) and *p*-cymene (4.7%) were found tobe the main compounds of *Grammosciadium platycarpum*; in another study with *Grammosciadium macrodon* contained thirty-seven components representing 94.4% of the total oil, with caryophyllene oxide (15.9 %), germacrene D (12.4 %), β-caryophyllene (11.5 %) and δ-3 - carene (10.1%) as the main constituents [24]. In this study with *Grammosciadium macrodon* caryophyllene oxide (12.5%), camphor (11.8%), β-caryophyllene (7.2%) and□□-3-carene (10.4%) were determined as major compounds; whereas germacrene D determined only low amounts (Table 1). The essential oil of aerial parts of *Grammosciadium scabridum* was characterized high amounts of δ-terpinene (73.5%), *p*-cymene (14.2%), β-farnesene (5.3%) [25].

Borneol (26.4%), □-caryophyllene (15.3%), *p*-cymene (10.2%) and bornyl acetate (8.9%) were identified major components of *Inula macrocephala* [26]; in this study □-caryophyllene (20.4%), germacrene D (11.4%) and borneol (11.3%) were detected major constituents of *Inula germanica* (Table 1). In a study the essential oil composition aerial parts of *Tanacetum heterotomum*, *Tanacetum zahlbruckneri*, *Tanacetum densum* subsp. *amani* and *Tanacetum cadmeum* subsp. *orientale* were analyzed; germacrene D (22.4%), borneol (18.8%) and spathulenol (17.9%) were detected the main constituents of *T. heterotomum;* germacrene D (21.4%), borneol (21.3%) and spathulenol (16.2%) were determined the main components of *Tanacetum zahlbruckneri;* camphor (26.8%), 1,8-cineole (16.7%), □-pinene (18.8%), α-pinene (6.7%) were found to be the main constituents of *Tanacetum densum* subsp. *amani*; 1,8-cineole (19.6%), camphor (17.2%), α-pinene (18.8%) were reported the major constituents of *Tanacetum cadmeum* subsp. *orientale* [27]. In this research borneol (20.7%), 1-8 cineole (13.4%) and camphor (12.4%) were detected main compounds of *T. densum* subsp. *eginense* (Table 1). In the *Tanacetum zahlbruckneri* palmitic acid, linoleic acid and α-linolenic acid was found as the main fatty acids [28]. In this study palmitic (21.89%), stearic (11.96%), linoleic (14.12%), linolenic acid (35.25%) were found to be the main fatty acid composition of *Tanacetum densum* subsp. *eginense* (Table 2).

Inconclusion, some qualitative and quantitative differences were detected between studied species in view of main compounds. These variations depending on genetic, environmental factors, ontogeny, season, plant part analyzed, analytical methods or defence and protection from insects, animals or pathogens. The findings showed that the studied plant taxa had some variations in fatty acid and essential oil composition. Detected main compounds can be chemotaxonomic marker of studied samples.

Table 1: Essential oil compounds of studied samples (%).

Compounds	*RRI	Tanacetum densum subsp. eginense	Inula germanica	Grammosciadum macrodon	Seseli peucedanoides
Santolinatriene	998	0.3	0.6	0.4	-
Tricyclene	1012	0.1	-	0.2	-
□-thujene	1016	1.1	1.2	-	0.5
□-pinene	1023	3.4	1.1	1.7	12.6
Camphene	1035	1.2	2.3	1.5	-
Sabinene	1054	-	0.6	-	8.2
□-pinene	1059	3.5	0.4	3.9	4.8
Benzene	1071	0.2	0.2	-	-
p-cymene	1080		8.5		
□-phellandrene	1082	0.1	-	0.2	11.1
□-terpinene	1085	0.3	0.4	-	0.5
Limonene	1094	1.6	0.6	8.9	21.2
1,8-cineole	1101	13.4	2.3	2.7	-
trans-sabinenehydrate	1124	0.6	-	0.1	-
□-3-carene	1130	-	-	10.4	0.4
□-terpinolene	1135	2.2	0.9	-	-
Cyclohexene	1145	-	-	1.2	0.7
Cis-sabinenehydrate	1151	0.2	-	1.3	-
Trans-chrysanthemol	1168	0.1	2.7	-	3.1
4-acetyl-1- ethylcyclohexan	1171	0.6	-	1.2	0.6
Trans-pinocarveol	1178	-	0.3	-	-
Camphor	1182	12.4	4.8	11.8	-
Pinocarvone	1190	1.0	-	2.4	1.8
2-cyclohexen-1-ol	1203	-	-	0.6	-
Borneol	1208	20.7	11.3	2.8	5.3
3-cyclohexen-1-ol	1211	0.2	0.4	-	0.4
□-terpineol	1218	0.3	2.9	0.3	-
Trans-carveol	1231	0.4	0.1	0.7	0.4
Propanoic acid	1246	0.1	0.2	-	-
Trans-geraniol	1256	-	-	0.6	0.5
Bicyclo (2.2.1) heptan-2-ol	1282	0.2	1.1	-	-
Thymol	1285	-	0.4	0.3	0.2
Phenol	1291	0.6	-	-	0.5
□-terpinene	1332	0.4	0.8	0.2	-
Cis-verbenol	1353	-	-	-	0.4
□-copaene	1360	1.3	-	2.6	2.8
□-bourbenene	1371	0.8	1.5	1.4	-
□-elemene	1374	-	-	-	1.9
□-caryophyllene	1385	0.5	20.4	7.2	-
□-cubebene	1396	1.2	0.9	0.4	1.3
Trans-□-farnesene	1412	-	-	1.2	-

Ledene	1420	0.4	0.5	-	1.5
Germacrene D	1432	12.8	11.4	3.9	5.8
□-selinene	1442	-	1.1	5.3	-
Naphthalene	1453	0.4	-	0.8	0.4
□-cadinene	1460	-	-	-	0.3
Spathulenol	1495	0.9	6.2	1.4	-
Caryophylleneoxide	1500	2.1	3.2	12.5	0.2
Muurolene	1518	0.4	-	0.2	1.6
Copaene	1539	1.4	0.8	0.3	-
□-cadinol	1541	-	1.1	-	1.3
□-bisabolene	1551	0.7	-	1.1	0.8
Aromadendrene	1558	0.4	1.6	-	1.3
2-pentadecanone	1625	-	0.4	0.2	-
Hexadecanoic acid	1685	0.6	-	0.3	0.7
Carene	1741	0.3	0.7	-	0.4
Octadecanal	1862	-	-	0.1	0.5
Tricosane	1902	0.7	1.5	-	-
Eicosane	1935	1.4	-	0.2	0.6
*RRI: Relative Retention Index	Total	91.5	94.4	92.3	93.5

Table 2: Leaf fatty acid composition of studied samples (%).

	C 12:0	C 14:0	C 16:0	C 17:0	C 18:0	C 18:1	C 18:2	C 18:3	C 20:3	C 22:0	C 24:0
Tanacetum densum subsp. eginense	0.00	3.42	21.89	2.51	11.96	9.26	14.12	35.25	0.00	0.00	1.58
Inula germanica	4.33	6.48	31.72	0.00	18.18	4.89	20.47	13.93	0.00	0.00	0.00
Grammasciadium macrodon	0.00	0.00	30.62	0.00	19.88	15.74	33.77	0.00	0.00	0.00	0.00
Seseli peucedanoides	0.00	2.16	47.48	4.66	31.09	2.91	6.46	5.24	0.00	0.00	0.00

*Lauric acid methyl ester (C12:0); Myristic acid methyl ester (C14:0); Palmitic acid methyl ester (C16:0); Heptadecanoic acid methyl ester (C17:0); Stearic acid methyl ester (C18:0); Petroselinic acid (C18:1); Linoleic acid methyl ester (C18:2); Linolenic acid methyl ester (C18:3); Mead acid (C20:3); Behenic acid methyl ester (C22:0); Tetracosanoic acid (C24:0).

Acknowledgments

The author thank the financial support from the Bingol University Scientific Research Project Unit, Bingol/Turkey, Project no. Pikom-Bitki-2018.003.

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