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Essential oil composition of two *Prangos* Lindl. (Apiaceae) species from Turkey

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Summary. The essential oil of the dried flowering aerial parts of *Prangos pabularia* Lindl. and *Prangos peucedanifolia* Fenzl. were analyzed by means of HS-SPME/GC-MS. As a result thirty four and thirty seven components were identified representing 91.3% and 89.5% of the oil of *P. pabularia* and *P. peucedanifolia*, respectively. The main constituents of *P. pabularia* were α-pinene (32.4%), δ-3-carene (12.4%), germacrene D (8.1%), limonene (6.4%) and bicyclogermacrene (6.2%); whereas α-pinene (38.1%), bicyclogermacrene (11.3%) and δ-3-carene (9.2%) were the major constituents of *P. peucedanifolia.* With this study, chemotypes of *P. pabularia* and *P. peucedanifolia* were detected α-pinene and δ-3-carene. Studied plant samples were found to be rich in respect to essential oils. The results were discussed in consideration of natural products, renewable resources, chemotaxonomy and potential medical uses of these plants.

Key words: *Prangos*, essential oil, HS-SPME/GC-MS

Introduction

Plants breed a variety of volatile, lipophilic substances known as essential oils that include mainly hydrocarbons or monofunctional compounds derived from metabolism of mono and sesquiterpenes, phenylpropanoids, amino as well as fatty acids (1). Essential oils have a complex composition, containing from a few dozen to several hundred constituents. Especially hydrocarbons and oxygenated compounds are responsible for the characteristic odours and flavours (2). Essential oils are compounds made of several organic volatile substances and they are produced and stored in the secretion canals of plants. At room temperature essential oils are usually liquid and they are responsible for the aromas of plants. Essential oils are widely distributed in nature and are found in conifers, Myrtaceae, Rutaceae, although the majority of plants with essential oils are found in the Lamiaceae and Apiaceae families (3). Essential oils can be extracted from plant materials by several methods, among all methods steam distillation has been widely used, especially for commercial scale production (4). Essential oils are found in different organs: roots, ryzomes, wood, leaf and flowering parts. The composition depends on place of origin. The habitat where the plant grows (normally warm climates have more essential oils), the moment of harvesting, extraction methods, etc… are also important. Among the main therapeutic properties of essential oils antiseptics stands out (for many years these spices have been added to foodstuffs not just for flavouring but to help preserve them). Other properties are: antispasmodic, expectorant, carminative, eupeptic, anticancer, antimicrobial, antioxidant, and usage as free radical scavenging agents (5, 6). Because of antioxidant and antimicrobial activities, essential oils are serving as natural additives in foods and food products (7). Because of the increasing attention in natural additives, essential oils of plants have been used more widely, thus, essential oils can serve as the alternative additives or processing aid as green technology, phytoteraphy and aromateraphy. We should bear in mind that certain essential oils, especially in high doses, may be toxic to the central nervous system in particular. Others, such as rue or juniper have abortive properties. Others may cause skin problems, rashes or allergies In addition to having therapeutic properties, essential oils are widely used in the food, pharmaceutical, cosmetic, veterinary products, textile, tobacco, biocides and insecticides industries (8).

Although essential oil constituents are thought to be end products of metabolism, it has become evident that they may have some ecological roles in plants. Essential oil compounds may play an attractant role for insects in pollination and in the seed dispersing process (9). A wide variety of essential oils are known to possess antifungal and antibacterial features, serving as chemical defense agents against plant pathogens (10). They exhibit cytotoxic effects being able to act as anti herbivorous agents (11). In arid and semi-arid areas during the summer time, essential oil constituents were found to be released from the dominant plants, suppressing the growth of herbaceous ones around them that tend to compete with the dominant plants in water and nutrients uptake; so it becomes evident that the essential oils could serve as allelopathic agents in plants (12). The genus *Prangos* Lindl. is in the Apiaceae (Umbellifera) family; comprises 43 taxa in the worldwide where it is primarily characterized by central and west Asia and Mediterranean regions. In the Flora of Turkey *Prangos* is an important genus and has 16 taxa recorded in Turkey (13-15). The *Prangos* has a long historical medical record starting from De Materia Medica by Dioscorides. *P. ferulacea* (L.) Lindl. was recorded under the name "Ippomarathon", for the treatment of the kidney and the urinary tract diseases (16). In Turkey *Prangos ferulacea* and *Prangos pabularia* are the most widespread species; they both are locally known as "Casir" or Caksir", and their decoctions are used as stimulants (17). Roots of *Prangos* taxa are also used in Turkey as aphrodisiac like *Ferula* and *Ferulago* species (18). As external application, *P. platychlaena* Boiss. et Tchihat. has been used to stop bleeding in skin or to heal the scars in eastern Turkey (19). In Iran, *P. pabularia* called "Djashire-Ulufei" and root extracts have been used as diuretic (20). *Prangos pabularia* spread further to central Asia and fruits were used as carminative, stimulant and diuretic in Amhi system which is a traditional healing system in Himalayas (21). The roots of *P. pabularia* have been used for emmenagogue in India (22). *P. peucedanifolia* which is locally known as "Karkol" has been used for treating kidney disorders, bladder inflammation and hemorrhoids in Iraq (23). An infusion of aerial parts of *P. asperula* Boiss. has been recorded to treat reduced skin disease, blood pressure and digestive diseases in Lebanon (24). In Uzbekistan *Prangos tschimganica* B. Fedtsch. has been used as a folk medicine for cure of leukoplakic disease (25). Literature survey revealed that alkaloids, coumarins, flavonoids and terpenoids were isolated from various *Prangos* taxa. *Prangos* extracts and some of isolated compounds have been studied for biological activities including antibacterial, antispasmodic , anti-inflammatory , antioxidant and cytotoxic effects (26, 27). According to literature records, the diversity of the oils and the chemical differences among *Prangos* can be the consequence of the diverse geographical conditions, genetic factors and distillation techniques (28, 29).

This study is a part of our ongoing research project on phytochemical investigation of medicinal and aromatic plants. In this study chemical and medicinal properties of *P. pabularia* and *P. peucedanifolia* are the subject of a detailed research. To the best of our knowledge, the essential oil of the aerial parts at flowering stage of these plants in Bingol area from east part of Turkey have not been investigated before. Therefore, the aim of this study is to provide essential oil composition of these species, that might be helpful in potential usefulness, biological activities, chemotaxonomy and other studies with *Prangos* taxa.

Materials and Methods

Plant materials

Prangos pabularia was collected from south of Güneytepe village, steppe, inclined areas Elazığ-Keban / Turkey, on 13.06.2015, at an altidude of 1250-1300 m., by O. Kilic, collect no: 5792. *P. peucedanifolia* was collected from east of Denizli village, moisty and stony areas, Elazığ-Keban / Turkey, on 13.06.2015, at an altidude of 1000-1100 m., by O. Kilic, collect no: 5796. Plant materials were identified with volume 7 of Flora of Turkey and East Aegean Islands (30). Voucher specimens were deposited in the Bingol University, Department of Park and Garden Plants and Yildirimli herbarium from Ankara.

HS-SPME procedure

Dried aerial part powder of five grams plant samples were carried out by a (HS-SPME) head space solid phase microextraction method using a divinyl benzene/carboxen/ polydimethylsiloxane fiber, with 50/30 µm film thickness; before the analysis the fiber was conditioned in the injection port of the gas chromatography (GC) as indicated by the manufacturer. For each sample, 5 g of previously homogenized plant sample was weighed into a 40 ml vial; the vial was equipped with a "mininert" valve. The vial was kept at 35°C with continuous internal stirring and the sample was left to equilibrate for 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35°C. After sampling, the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analytes thermal desorption. In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time were studied on the extraction efficiency as previously reported by Verzera et al. (31).

GC-MS analysis

A Varian 3800 gas chromatograph directly interfaced with a Varian 2000 ion trap mass spectrometer was used with injector temperature, 260°C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 lm film thickness. The oven temperature was programmed as follows: 45°C held for 5 min, then increased to 80°C at a rate of 10°C/min, and to 240°C at 2°C/min. The carrier gas was helium, used at a constant pressure of 10 psi; the transfer line temperature, 250°C; the ionisation mode, electron impact (EI); acquisit ion range, 40 to 200 m/z; scan rate, 1μs^{-1}. The compounds were identified using the NIST library, mass spectral library and verified by the retention indices which were calculated as described by Van den Dool and Kratz (32).

The relative amounts were calculated on the basis of peak-area ratios. The identified constituents of studied species are listed in Table 1.

Results and Discussion

The essential oil aerial parts of *P. pabularia* and *P. peucedanifolia* were analyzed by means of HS-SPME/GC-MS. As a result thirty four and thirty seven components were identified representing 91.3% and 89.5% of the oil of *P. pabularia* and *P. peucedanifolia*, respectively. The main constituents of *P. pabularia* were *α-pinene* (32.4%), *δ*-3-carene (12.4%), germacrene D (8.1%), limonene (6.4%) and bicyclogermacrene (6.2%); whereas *α*-pinene (38.1%), bicyclogermacrene (11.3%) and *δ*-3-carene (9.2%) were the major constituents of *P. peucedanifolia*. Among the monoterpenes, α-pinene was found principal constituents of *P. pabularia* (32.4%) and *P. peucedanifolia* (38.1%) (Table 1).

In a study analysing the volatile constituents of *Prangos acaulis* (DC) Bornm from Iran, the major compounds of the oil were δ-3-carene (25.54%), α-pinene (13.6%), α-terpinolene (14.76%), limonene (12.94%) and myrcene (8.1%) (33). In this study, *P. pabularia* and *P. peucedanifolia* have similar essential oil composition properties and are different from the cited study, producing high concentration of α-pinene (32.4%-38.1%) and δ-3-carene (12.4%-9.2%); followed by low percentages of terpinolene and myrcene respectively (Table 1). A comparison of the essential oil composition of *P. pabularia* and *P. peucedanifolia* with that of some other taxa of the genus *Prangos* in the literature shows that considerable differences exist in the compositions of the essential oils of different species of the genus *Prangos*, especially in terms of the type of major components. It has been determined that the most abundant component in the essential oils of *P. uloptera*, *P. bornmuelleri*, *P. heyniae*, *P. ferulaveae* and *P. uechtritzii* were α-pinene (15.0%), δ-3-carene (16.1%), germacrene D (42%), β-bisabolene (53.3%), γ-terpinene (27.8%), and *p*-cymene (10.9%) respectively (34-37). Similarly α-pinene and δ-3-carene were the main components of *P. pabularia* and *P. peucedanifolia* (Table 1). However, α- and β-pinene, germacrene D, bicyclogermacrene, limonene and δ-3-carene are of

Table 1. Essential oil composition of *Prangos* species (%)

Compounds	**RRI***	***P. pabularia***	***P. peucedanifolia***
Hexanal	871	0.2	-
Heptanal	905	-	0.3
α-Thujene	927	1.2	0.6
α-Pinene	935	32.4	38.1
Camphene	948	-	0.1
Sabinene	965	0.2	-
β-Pinene	975	0.8	1.2
3-Octanone	981	-	0.4
β-Myrcene	988	1.6	1.2
α-phellandrene	995	0.2	0.3
δ-3-carene	1002	12.4	9.2
3-Octanol	1006	-	0.1
α-Terpinene	1015	0.5	-
p-Cymene	1023	-	0.2
β-phellandrene	1028	2.7	1.4
β-ocimene	1030	-	0.1
Limonene	1033	6.4	2.3
1,8-Cineole	1042	0.1	-
γ-Terpinene	1062	1.1	1.3
Sabinene hydrate	1070	-	0.2
Terpinolene	1082	0.6	-
Linalool	1085	0.1	-
Nonanal	1098	-	0.2
Terpinen-4-ol	1125	0.2	-
Trans verbenol	1142	0.3	0.1
n-Decanal	1205	-	0.4
Cis-chrysanthenyl acetate	1255	1.4	1.2
Trans-Anethol	1280	4.2	3.8
Bornyl acetate	1295	2.2	-
Bicycloelemene	1330	-	0.4
α-Copaene	1352	0.1	-
α-Ylangene	1372	0.2	0.3
β-Bourbonene	1382	-	0.1
β-Elemene	1395	0.2	-
Methyl eugenol	1400	-	0.2
β-Cubebene	1402	-	0.3
β-Caryophyllene	1412	0.1	-
γ-elemene	1428	0.4	0.2
α-Humulene	1450	1.4	4.2
β-farnesene	1460	-	0.2
Germacrene D	1476	8.1	3.2
Bicyclogermacrene	1495	6.2	11.3
β-selinene	1500	-	0.2
α-muuorelene	1505	0.1	-
β-Bisabolene	1513	1.2	2.3
β-Farnesene	1520	-	0.3
γ-Cadinene	1526	0.1	1.2
Spathulenol	1572	0.2	-
Germacrene B	1595	3.1	2.1
Cadinol	1635	1.1	-
Heptadecane	1645	-	0.3
	Total	**91.3**	**89.5**

RRI*: Relative Retention Index

common occurrence in the essential oils of fruits of nearly all investigated *Prangos* taxa.

There are some studies on essential oils of different parts of *P. ferulacea* from various locations; these results suggest that α-pinene and β-pinene are the dominant constituents in the different plant parts, although there were some differences observed in the essential oil profiles in different habitats (38-39). E-anethole was the main constituent at the flowering stage (as high as 95.5% of the oil), whereas it was found in low quantities at the vegetative stage. It is reported in literature that anethole has antiproliferative and mosquitocidal activity (1). Anethole was also identified as the most active fumigant against all pest species on the basis of activity at a given dose and exposure period (11). Therefore, it is assumed that fungitoxic and phytotoxic effects of *P. ferulacea* leaves at flowering stage might be attributed to the presence of anethole (40). According to the some work in the literature, essential oils of the Apiaceae plant's umbels and fruits were dominated by α-pinene and β-pinene, beside other monoterpenes. They play an attractant role for insects during pollination and seed dispersal period. It is suggested that biosynthetic pathways shift to produce some allochemicals like anethole during the reproductive stage. This change may protect a plant against herbivorous insects. *P. pabularia* and *P. peucedanifolia* were dominated by α-pinene and *trans*-anethole (Table 1), so these plant's umbels and fruits can have an attractant role for insects during pollination and against herbivorous insects.

In another research, the hydrodistilled oil from crushed dry fruits of *Prangos asperula* Boiss. subsp. *haussknechtii* (Boiss.) Herrnst. et Heyn which is grown wildly in Iran was analyzed by GC/MS and fifty-two constituents were identified of which δ-3-carene (16.1%), β-phellandrene (14.7%), α-pinene (10.5%), α-humulene (7.8%), germacrene-D (5.4%), δ-cadinene (4.2%) and terpinolene (4.0%) were found to be the main constituents of the oil (41). In this study, main constituents of *P. pabularia* and *P. peucedanifolia* were α-pinene and δ-3-carene. It is noteworthy that β-phellandrene, α-humulene and terpinolene were found to be low percentages in this research (Table 1). Details of essential oil compositions of *P. pabularia* and *P. peucedanifolia* oils can be seen in Table 1. Comparison of the oil compositions from two species showed that the amounts of δ-3-carene and germacrene-D were higher in the oil of sample *P. pabularia* than *P. Peucedanifolia*. Another difference is the lower amount of bicyclogermacrene in *P. pabularia* (6.2%) than *P. peucedanifolia* (11.3%) (Table 1). The percentage of some minor components in both oils were similar, but there are some differences between the percentages of some other components: For example, limonene, *trans*-anethole, sabinene, β-myrcene, germacrene D, α-humulene and so on (Table 1). These differences can be the result of different ecological properties and might have been derived from local, climatic factors of two plant localities. Of course *P. pabularia* than *P. peucedanifolia* oils were very similar in the minor components, especially in monoterpenoid compounds (Table 1).

The water-distilled essential oils from crushed dry fruits of *Prangos heyniae*, a recently described endemic plant in Turkey, collected from two localities, were analysed and the oils were characterized as β-bisabolenal (53.3% and 18.0%), β-bisabolenol (14.6% and 2.3%) and β-bisabolene (12.1% and 10.1%) as main constituents. Germacrene D (13.5%) and germacrene B (9.4%) were also main components in one of the samples (42). It is noteworthy that in this research β-bisabolenal and β-bisabolenol were not detected and β-bisabolene (1.2% and 2.3% respectively) was in a lower percentage in *P. pabularia* than *P. peucedanifolia* (Table 1). A detailed analysis of *Prangos pabularia* fruit oil was performed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS): Bicyclogermacrene (21%), (*Z*)-β-ocimene (19%), α-humulene (8%), α-pinene (8%) and spathulenol (6%) were the main compounds of the oil (43). Also in our research we found that α-pinene and bicyclogermacrene were the main compounds both oils (Table 1). Hovewer β-ocimene, α-humulene and spathulenol were detected either in low amounts or not at all in *P. pabularia* and *P. peucedanifolia* oils (Table 1). The essential oil composition of various *Prangos* taxa have been investigated from diverse locations, different plant tissues and different extraction techniques that influenced their essential oil profile (34-36). *Prangos pabularia* fruit oil grown in Iran contained mainly α-pinene 33.87%, spathulenol 9.32% and α-santalone 7.05% (40). We obtained similar results in our research: α-pinene was the main compound of *P. pabularia* and *P. peucedanifolia* (32.4% - 38.1% respectively) . But spathulenol and α-santalone were found in lower or no amounts in our samples (Table 1). This difference can be expected due to difference in locations where samples were collected.

In conclusion, this study demonstrates the occurrence of α-pinene (32.4%), δ-3-carene, bicyclogermacrene chemotypes of *P. pabularia* and *P. peucedanifolia* in Eastern Anatolian region of Turkey. The essential oil results have given some clues on the chemotaxonomy of the genus patterns and usability of *P. pabularia* and *P. peucedanifolia* as natural product. According to these results, studied plants were found to be rich in respect to essential oils. So these plants can be used different purposes in industry and ethnobotany. They can be cultivated to richen natural products and to develop chemicals against insect pest. In addition, many plant species are threatened due to overharvesting for medicinal or other use, so there is great need to protect plant diversity. There is also a need to develop more sustainable ways of obtaining industrial products from renewable resources. The cultivation of medicinal and aromatic plants for industrial products can address these issues. Furthermore, the results showed that the analysis of essential oil composition will add some contributions on the usability of this plant as a crop. The results will also help in the chemotaxonomy of the genus patterns.

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