Research article
Curr. Pers. MAPs, 2019; 2(2): 125-132

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Determination of Essential Oil Content and Composition, Total Phenolic Content and Antioxidant Activities of *Pastinaca sativa* L. subsp *urens* (Req. Ex Gordon)

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Received : 25/11/2019
Accepted : 21/12/2019

Abstract

This study was carried out in 2017 in order to determine the essential oil composition, total phenolic contents and antioxidant activity of fruits and above-ground parts of *Pastinaca sativa* L. subsp *urens* (Req. Ex Gordon) Celak, which grow naturally in the flora of Göller Region, Turkey. Plant samples were collected at full flowering and fruit samples were collected at theyellow maturing period. The essential oils were obtained by hydro-distillation and components of the oils were identified by gas chromatography/mass spectrometry. Methanol extracts of fruit and plant samples were used for total phenolic content and antioxidant activity. Total phenolic contents were 50.40 ± 1.40 mg/gr and 67.86 ± 1.02 mg/gr in the fruit and herb samples, respectively. Antioxidant activity of the herb extract was higher than the fruits. Essential oil contents of fruit and herb samples were 3.20% and 0.33% and the numbers of components forming essential oils were 28 and 29, respectively. There were 47 different components identified in total for fruit and herb samples. The main component of the fruit oil was octyl butyrate (90.4%), while cis-ocimen (38.2%), Furanone (14.1%), octyl Butyrate (13.2%) and Butanoic acid (11.1%) were the major components of the herba essential oil.

Key Words: *Pastinaca sativa* L. subsp*. urens,* Phenolic Content, Antioxidant Activity, Essential Oil Content and Component

1. Introduction

The genus *Pastinaca* (Apiaceae) is represented in Turkey by five species and altogether seven taxa: *Pastinaca sativa* L. subsp. *urens* (Req. ex Godron) Celak, *Pastinaca armena* Fisch. & Mey, *Pastinaca armena* Fisch. & Mey. subsp. *armena*, *Pastinaca armena* Fisch. & Mey. subsp. *dentata* (Freyn & Sint.) Chamberlain, *Pastinaca pimpinellifolia* Bieb., *Pastinaca zozimioides* Fenzl (endemic), *Pastinaca glandulosa* Boiss. & Hausskn. (Davis, 1972). The Pastinaca genus plants are perennial plants with small flowers and yellow fruits typical reprehensive of the Mediterranean flora (Davis, 1972). Different parts of *Pastinaca sativa* L. (Parsnip in English, Karakok, Kelemşir and Yabani havuc in Turkish) used for different purposes and, therefore, is widely cultivated and commercially traded in Europe (Baytop, 1999; Doğan, 2014). A whole plant is used for

strengthening the appetite, better digestion, and as a diuretic. The fruit is bitter aromatics that increases milk yield (Tucakov, 1986). The root is rich in starch and sugar and is used as food (parsnip), animal fodder, and for wine making. The sap is liable to cause skin irritation by sensitizing skin to UV radiation (Zehui and Watson, 2005). The seed is used as a condiment (Launert, 1981). It is similar in taste to dill (Facciola, 1990). Native parts of plants have been applied in feeding as a spice, pharmaceutical industry and folk medicine (Nikolić, 1973).

Above-ground parts, fruits and roots of *P. sativa* species contain essential oils of different properties. In the oil obtained from crushed seeds of *P. sativa* subsp. *urens* from Turkey, 18 components were characterized representing 95% of the oil with octyl butyrate (79.5%) and octyl hexanoate (5.3%) as the major constituents (Kurkcuoglu et al., 2006). The dominant constituents in fruit oil of *P. sativa* from Serbia were reported as myristicin (64.20%), *β*-ocimene (29.10%) and β-farnesene (24.50%) (Janciec et al., 1995). In the above-ground parts of *P sativa*, E-*β*-ocimene, Z-*β*-ocimene, *α*-terpinolene and E-*β*- farnesene has been identified as major compounds (Kubeczka and Stahl, 1977). The root essential oil has previously been reported to contain terpinolene (40–70%) and myristicin (17–40%) as the main constituents in the essential oil from fresh *P. sativa* roots (Stahl, 1975; Kubeczka and Stahl, 1975; Lawrence, 1979).

The occurrences of hydrocarbons and coumarins in the fruits were also reported (Brown et al., 1975; Stein and Posocco, 1984). Furanocoumarin compounds reported from the roots, stems, leaves, buds, flowers and fruits of cultivated and wild *P. sativa* included; angelicin, apterin, bergapten, imperatorin, isobergapten, isoimperatorin, isopimpinellin, pimpinellin, psoralen, sphondin, xanthotoxin and xanthotoxol (Pathak et al., 1962; Berenbaum, 1985; Berenbaum and Zangerl, 1986; Berenbaum et al., 1991). There is considerable variation in the toxicity and photoactivity of these different furanocoumarin compounds (Berenbaum et al., 1991). Considering the potential of essential oils and extracts for substituting synthetic antioxidants and preservers, the study of potential of *P. sativa* L. subsp *urens* (Req. Ex Gordon) can be useful for industrial application as a natural antioxidant sources.

To the best of our knowledge, there is no previous study on the antioxidant activity and phenolic content of fruits and above-ground parts of *P. sativa* species. The aim of this study was to evaluate and compare the essential oil content and constituents, total phenolic matter and antioxidant activities of methanol extracts from the fruits and aerial parts of *P. sativa* L. subsp *urens* (Req. Ex Gordon) from Turkey.

2. Materials And Methods

2.1. Plant Material

Fruits and above-ground parts of *P. sativa* L. subsp *urens* were collected by the authors from their natural habitats in Eğirdir, Isparta, Turkey in 2017 by random sampling from a single established population. All samples were collected at full flowering and fruit maturing stages for species identification and essential oil, antioxidant activity and total phenolic analyses. Plant materials were identified by Prof. Dr. Hasan ÖZÇELİK according to “Flora of Turkey” (Davis et al., 1988) and voucher specimens (63.73.1.1) were deposited in the Herbarium GUL, Suleyman Demirel University.

2.2. Essential oil analysis

Air-dried fruits (50 g) and fresh above-ground parts (500 g) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia (1980). The obtained oils were dried over anhydrous sodium sulfate

and stored in sealed vials at +4°C in the dark until chemical analyses.

2.3. Gas chromatography–mass spectrometry

GC–FID-MS analysis was performed on QP5050 GC-MS equipped with FID detector. The GC was equipped with CP-Wax 52 CB capillary column (50 m x 0.32 mm; film thickness = 0.25 µm) and helium was used carrier gas with flow rate of 1 mL/min. The GC oven was heated from 60°C to 230°C at a rate of 3°C/min, the final temperature was then maintained during for 20 min. The injector was maintained at a temperature of 250°C. Injection volume 0.1 mL of 1% solution prepared in n-hexane; split ratio 20:1. The mass spectrometer was operating in EI mode at 70 eV with mass scan range of 40–450 amu. Identification of constituents was done on the basis of RI (determined with reference to homologous series of n-alkanes C8–C25, under identical experimental condition), MS library search (NIST 08MS Library (Version 2.0 f) and Wiley MS 9th edition), and by comparison with MS literature data (Adams, 1995). The relative amounts of individual components were calculated based on GC peak area (FID response) without using the correction factor.

2.4. Determination of total phenolic content

Ground-dried above-ground parts and fruits (0.2 g) were extracted with 10 mL of 80% methanol at room temperature, using a magnetic stirrer for 15 min. After centrifugation for 10 min, the supernatant solution was filtered under vacuum into a volumetric flask. The residue was re-extracted in the same way and the final volume of the solution was set at 25 mL.The phenolic content of the fruit and above-ground parts of the species were made according to Singleton and Rossi (1965) using Folin-Ciocalte colorimetric method. The obtained results were read in a spectrophotometer at 765 nm wavelength and total phenolic contents were calculated as gallic acid equivalent in mg/g with the following equation.

Total Phenolic Content (mg/g)= [(reading * final volume) / volume in the pit) * (1 / weighing)]

2.5. Determination of antioxidant activity by DPPH method

The free radical trapping activity of fruits and above-ground parts were compared with synthetic antioxidants such as BHT (Butylated hydroxytoluene), BHA (Butylated hydroxyanisole) and Trolox [(±)-6-Hydroxy-2,5,7,8-tetramethylchromane -2-carboxylic acid] by DPPH (1,1-diphenyl-2-picryl-hydrazyl) method. (Shimada et al. 1992). Samples were prepared at 50, 100 and 250 ppm in 1 ml of methanol and 1 ml of 0.2 mM DPPH was added. The vortexed samples were incubated for 30 minutes at darkroom temperature and then measured on a spectrophotometer at a wavelength of 517 nm (PG Instruments T70 Plus Dual Beam Spectrophotometer, Arlington, MA, USA). The free radical trapping activity of the fruits and above-ground parts used in the study was determined by the following formula:

Antioxidant activity (%) = [(control abs - sample abs) / control abs] x 100

3. Results and Discussion

Fruits and above-ground parts of *P. sativa* L. subsp *urens* afforded yellowish oils, with yields (mean of three replicates) 3.20% and 0.11% (v/w), respectively. The GC-MS results of the essential oils are given in Table 1. A total of 39 compounds were identified which was 27 for fruits and 29 for above-ground parts representing more than 99% of the volatile fraction. Considering the different groups of compounds, a large part of the fruit oil was composed of esters (93.96%), whereas above-ground parts oils was formed monoterpene (49.55%) and esters (40.08%).

The total sesquiterpene content of the above-ground parts essential oils (4.74%) were higher than the fruits (0.37%) (Table 1). The components of both oils were substantially similar, but the proportions of the components were different. As shown in Table 1, the main compound in the essential oil from the fruits were octyl butyrate (90.43%), while these compound were found by 13.23% in above-ground parts oil. Hexyl butyrate (2.27%) and octyl hexanoate (2.87%) were the other important compounds found in fruit essential oils. On the other hand, Octyl Hexanoate was only found in the fruit essential oils. *Cis*-Ocimene (38.23%) predominates in the essential oil of above-ground parts and this compound present in very low concentrations in fruits essential oil (0.16%). Essential oils from the above-ground parts have a high level of octyl butyrate (13.23%) and butanoic acid (11.10%) which are found very low content in the oil from the fruits. Some components with content greater than 1% such as (X) *β*-ocimine, *α*-terpinolene and neo allo acimene were only found in the essential oil of above-ground parts.

There are a few pieces of researches have been reported on essential oil compounds of *P. sativa* in the literature (Kubeczka and Stahl, 1977; Kurkcuoğlu et al., 2006; Matejic et al., 2014). Fruit essential oil compositions *P. sativa* L. subsp *urens* from Turkey have been reported for the first time by Kurkcuoğlu et al., (2006). These researches reported that the main constituents of fruit essential oil of *P. sativa* L. subsp *urens* were octyl butyrate (79.5%) and octyl hexanoate (5.3%). In Kubeczka and Stahl (1977) study, herb oils from the wild *P. sativa* were characterized by the presence of octyl acetate. The other important constituents of the herb oils of wild *P. sativa* reported as cis *β*-ocimene, *trans*-*β*-ocimene, terpinolene and *trans*-P-farnesene (Kubeczka and Stahl, 1977). Similar to our findings, Matejic et al., (2014) reported that the essential oil content of aerial parts of *P. sativa* from the Belgrade was 1.00%. (Z)-*β*-ocimene (10.8%), hexyl butanoate (10.4%), (E)-*β*-farnesene (6.1%) and lavandulyl acetate (5.2%) were found as the main components in the same research. The major components of the composition of the essential oil German *P. sativa* were reported octyl butyrate (40.9%), octyl acetate (32.4%), hexyl butanoate (4.6%), Z-*β*-ocimene (4.3%), E-*β*-farnesene (3.4%) and *γ*-stearolactone (3.4%). According to the present results and previous reports the principal component is octyl butyrate. Octyl butyrate was also as the major constituents of the essential oils of Malabaila aurea (Vuckovic et al., 2014) and Heracleum sphondylium (Maggi et al., 2014) from Apiaceae. The genera *Pastinaca*, *Heracleum*, *Zosima* and *Tordylium* are characterized by the presence of octylesters and octanol in their essential oils (Özek et al., 2006; Figueiredo et al., 2008). These compounds, such as octyl acetate, octyl butyrate, octyl hexanoate, octyl octanoate and octyl isobutanoate, often constitute nearly the entire essential oil, with other compounds occurring only as minor ones (Chizzola, 2010).

Total phenolic analyses showed that the amount of extractable phenolic compounds in above ground part extract (67.86 ± 1.02 mg/g galic acid) is higher than that detected in fruit extracts (50.40 ± 1.40 mg/g galic acid). Numerous studies have been conducted on the phenolic contents of Apiaceae species, but no similar studies were found in *Pastinaca* species. Bagdassarian et al., (2013) reported that the phenolic contents of some Apiaceae species were highly variable and they found the phenolic contents of *Foeniculum vulgare*, *Anethum graveolens*, *Pimpinella anisum*, *Carum carvi* and *Coriandrum sativum* were 116, 70, 46, 26 and 17 mg GAE /100g, respectively. The methanol extract of *P. ferulacea,* a species of Apiaceae, herb' antioxidant activities was found to be 152 and the total phenolic

content 65.1 in the study carried out by Çoruh et al. (2007). Pandey et al. (2012) reported that phenolic content for methanol extracts of seven spices of Apiaceae ranged from 12.81 in *Carum carvi* to 45.26 mg RE/g extract in *C. sativum*.

Table 1. Percentage composition of the essential oil of the fruits and of above-ground parts Pastinaca *sativa* L. subsp *urens* (Req. Ex Gordon) Celak

RI	Components	Fruit	Above ground parts
1064	Sabinene	-	0.14
1069	*β*-Pinene	0.12	0.91
1074	Butyl Butanoic Acid	0.10	-
1079	*β*-Myrcene	0.47	1.09
1118	l-Limonene	0.02	0.16
1122	***Cis*-Ocimene**	**0.16**	**38.23**
1133	**(X) *β*-Ocimine**	**-**	**5.75**
1137	2-Methyl Butyl Butyrate	0.03	-
1149	Octilin	0.73	-
1176	*α*-Terpinolene	-	1.26
1180	Linalool	-	0.39
1215	Neo Allo Ocimene	-	1.62
1266	Hexenyl Butyrate	0.02	0.20
1274	**Hexyl Butyrate**	**2.27**	**1.01**
1284	*n*-Decanal	0.23	0.23
1293	Caprylyl acetate	0.06	0.20
1330	RT:16.792	-	0.10
1347	RT:17.325	-	0.14
1364	Anethole	0.06	-
1378	Heptyl Butyrate	0.02	-
1422	Benzyl Butyrate	0.08	0.77
1470	Octanol	0.45	-
1490	**Octyl Butyrate**	**90.43**	**13.23**
1496	*α*-Duprezianene	-	0.28
1515	*α*-Longipinene	-	0.21
1525	Benzyl Carbyl Butyrate	0.49	3.17
1531	*Trans*-Caryophyllene	0.13	2.62
1564	**Butanoic Acid**	**0.19**	**11.10**
1579	RT:24.317	-	0.67
1588	*α*-Curcumene	0.20	1.26
1590	*α*-Bergamotene	-	0.36
1606	Myristicin	0.06	-
1627	Isopulegyl Acetate	0.03	0.26
1637	*β*-Sesquiphellandrene	0.04	-
1645	Aromadendrene	-	0.29
1687	Dodecenylacetate	0.02	0.24
1693	**Octyl Hexanoate**	**2.87**	-
1699	Decyl Butyrate	0.62	-
2229	**Octadecanoic Acid**	**0.07**	**14.11**
Monoterpene		0.86	49.55
Ester		93.96	40.08
Sesquiterpene		0.37	4.74
Other		4.81	5.63
Total		100.0	100.0

DPPH is a stable free radical, widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (Hu et al 2004). The methanol extract of *P. sativa* fruits and above-ground parts and standard antioxidants (BHA, BHT and Trolox) DPPH radical scavenging activity shown in Table 2. According to the results, DPPH radical scavenging activity of fruit and above-ground parts extracts and BHT change depending on the using concentration. However, Trolox and BHA showed high levels of radical scavenging activity at a concentration of 50 ppm such as more than 94.5% and 86.6%, respectively. Antioxidant activity of plant extracts increased as the used concentration increased (Table 2). DPPH radical scavenging activity of the methanol extracts of above-ground parts were higher (more than two fold) than the fruit extracts. In addition, the above-ground parts extracts showed similar antioxidant activity to the BHA (Table 2).

Table 2. Antioxidant activity of fruits and above-ground parts of *Pastinaca sativa* L. subsp *urens* (Req. Ex Gordon) Celak

		Fruit	**Above ground parts**
P. sativa L. subsp *urens*	250 ppm	24.71±0.46	55.54±2.6
	100 ppm	8.26±0.56	33.81±1.9
	50 ppm	3.28±0.18	23.1±2.3
Trolox	250 ppm		95.1±0.9
	100 ppm		94.7±0.9
	50 ppm		94.5±1.3
BHT	250 ppm		52.6±0.7
	100 ppm		34.9±1.5
	50 ppm		26.0±1.6
BHA	250 ppm		88.2±1.3
	100 ppm		88.0±0.2
	50 ppm		86.4±0.6

The natural antioxidants in plants have a great interest in natural product science, and many herbs have significant antioxidant potency (Ngi et al., 2000). Antioxidants decrease oxidative stress in cells and are therefore very useful in the treatment of many diseases and protect to plant many stress factors (Krishnaiah et al., 2011). The physiological role of antioxidant compounds is to scavenge for free radicals (Muraina et al., 2009; Halliwell and Gutterigde, 1989) in case of the overproduction of these reactive species (Wong et al., 2006). Phenolic compounds inhibited MDA concentration during lipid peroxidation; thus, they exhibited antioxidant activity. Methanol extracts contain both the nonpolar and polar compounds (aglycones and glycosides) in the plant. High correlation was reported between the antioxidant capacity and total phenol and flavonoids contents of plants (Wong et al., 2006; Leong and Shui, 2002). Besides antioxidant capacity, phenolic compounds exhibit a wide range of biological activities, including anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, anti-allergenic, anti-microbial and anti-stress (Hu et al., 2004). It is well known that plant phenolics, in general, are the highly effective free radical scavenging and antioxidants.

4. Conclusion

This study provides useful information on the fruit essentail oil quality and quantity as well as the fenolic content and antioxidant activity of the *P. sativa* subsp. *urens*. The majority of

the essential oil components were esters for furits and monoternes and esters for above-ground parts. Fruits of the specie may be valuable for pharmacological applications due to high essential oil and ester content and above-ground parts of the specie can be evaluated for exploitation in pharmaceutical and food industries due to its moderate antioxidant activity.

Acknowledgements

The authors are grateful to Dr. Hasan ÖZÇELİK from Süleyman Demirel University Biology Department for identifying the plants, to TUBİTAK (Turkey Bilimsel ve Teknolojik Araştırma Kurumu, grant number: 113O284) for financial support. This study is derived from PhD thesis named "Göller Yöresinde Yayılış Gösteren Apiaceae Familyasına Ait Bazı Bitki Türlerinin Ekonomik Değerleri ile Kültüre Alma Potansiyellerinin Belirlenmesi".

Conflict of Interest

The authors decleared that no conflict of interest.

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