Composition of essential oil and fatty acids and antioxidant assays of *Psephellus pyrrhoblepharus* (Boiss.) Wagenitz

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In this study, the essential oil of *Psephellus pyrrhoblepharus* (Boiss.) Wagenitz was obtained by hydrodistillation using a Clevenger-type apparatus. The oil was analysed by GC and GC/MS system. The components were separated as for adherence to the column and evaluated as for relative rate. Mass spectra of each component were taken. GC and GC/MS analyses of the essential oil from *P. pyrrhoblepharus* determined the identification of 59 components representing 84% of the oil. With this analysis, the major component was found as hexadecanoic acid (17.1%). Subsequent to this component, other major components were spatulenol (10.3%), caryophyllene oxide (9.6%) and heptacosane (5.2%). In addition to this, fatty acid methyl esters (FAMEs) from *P. pyrrhoblepharus* were prepared for analysis of fatty acids. By this test the amount of saturated fatty acids (SFA) were 31.37% with major fatty acid palmitic acid (12.86%). Also, the antioxidant assays DPPH, ABTS, ORAC, CUPRAC and reducing power assay were carried on methanol:water, n-hexane, chloroform and ethyl acetate extracts of *P. pyrrhoblepharus*. With the assays, ethyl acetate extract of *P. pyrrhoblepharus* was found as the most antioxidant extract. These data show that the plant is valuable for further studies.

Keywords: *Psephellus pyrrhoblepharus*, essential oil, fatty acids, antioxidant assay.

INTRODUCTION

The genus *Psephellus* Cass. (Asteraceae) has approximately 80 species. In Turkey, this genus is represented by 32 species with the species which are added in recent years. 12 sections which were in the genus *Centaurea* were transferred into the genus *Psephellus*; *P.* sect. *Psephelloidei* (Boiss.), *P.* sect. *Psephellus* (Cass.), *P.* sect. *Hyalinella* (Tzvelev), *P.* sect. *Aetheopappus* (Cass.), *P.* sect. *Amblyopogon* (DC.), *P.* sect. *Heterolophus* (Cass.), *P.* sect. *Czerniakovskya* (Czerep.), *P.* sect. *Odontolophoidei* (Tzvelev), *P.* sect. *Odontolophus* (Cass.), *P.* sect. *Xanthopsis* (DC.), *P.* sect. *Uralepis* (DC.), *P.* sect. *Sosnowskya* (Takht.). The plant *Psephellus pyrrhoblepharus* (Boiss.) Wagenitz is in the section *Psephelloidei* (Boiss.) [1-3].

Previous phytochemical investigations on this genus generally revealed the isolation of sesquiterpene lactones, flavonoids and lignans. For some plants in *Psephellus* genus have showed anti-inflammatory activity. Some studies with species in the genus *Centaurea* have shown that they have cytotoxic, antibacterial, antipyretic, antioxidant and antimicrobial activity. The essential oil compositions of some *Centaurea* species from Turkey have been investigated. Generally, germacrene D, hexadecanoic acid, caryophyllene and caryophyllene oxide were reported to be the major volatile components in the earlier studies [4-6]. The composition of essential oil of *Psephellus pyrrhoblepharus* was reported in a previous study [7]. For fatty acid compositions of some

Centaurea species in previous investigations there have been reported for major fatty acids as linoleic acid, palmitic acid, oleic acid. There have been no reports regarding to fatty acid composition of *P. pyrrhoblepharus.*
In this study, the essential oil and fatty acid compositions of *P. pyrrhoblepharus* were examined. Antioxidant activities of methanol:water (1:1), chloroform, n-hexane and ethyl acetate extracts from the aerial parts of *P. pyrrhoblepharus* were also studied. This is the first report on the fatty acid composition of *Psephellus pyrrhoblepharus* together with antioxidant activities of four different extracts of the plant.

MATERIALS AND METHODS

PLANT MATERIAL

Psephellus pyrrhoblepharus (Boiss.) Wagenitz was collected during flowering period from rocky areas of Buzluk Cave, Harput, Elazığ in 2012, 1550 m. This plant was identified by Assoc. Prof. Ugur Cakilcioglu from Munzur University, Tunceli. The plant was stored under number 1464 in the Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

ESSENTIAL OIL EXTRACTION

Air-dried aerial parts of the plant materials were subjected to hydrodistillation using a Clevenger-type apparatus for 8 h to yield according to the method recommended in the Pharmacopoeia [8]. The essential oil of the plant was obtained by yield of 0.01%. The oil was stored at +4°C in the dark until tests. Besides, water:methanol (1:1), chloroform, ethyl acetate and n-hexane extracts of the plant were prepared for the antioxidant activity assays.

GAS CHROMATOGRAPHY ANALYSIS OF ESSENTIAL OIL

The essential oil of the plant was analysed with the method GC/MS using HP Agilent 5975 GC-MSD in Anadolu University. Helium was used as the carrier gas. Injector temperature was 250ºC with 0.8 ml/min split flow. Temperature of the GC oven was kept at 60ºC for 10 min and then programmed to 220ºC with an accelerating rate of 4ºC /min. After that it was kept at 220ºC for 10 min and then programmed at 240ºC with an accelerating rate of 1ºC/min. The rate of split was 40:1 and MS were done at 70 eV. The range of mass was 35-450.
The GC analysis was carried out by using HP Agilent 6890N GC equipped with a FID detector and HP-Innowax Fased Silica Capillary Column (FSC, 60 m × 0.25 mm and film thickness 0.25 µm). To obtain the same elution order with GC/MS, simultaneous auto-injection was performed on a duplicate of the same column applying the same operational conditions. The components were separated as for adherence to Innowax FSC column and evaluated as for relative rate. Mass spectra of each component were taken. The relative percentages of the separated compounds were calculated from FID chromatograms.

GAS CHROMATOGRAPHY ANALYSIS OF FATTY ACIDS

Air-dried aerial parts of the plant materials were subjected to Soxhlet extraction at 60ºC for 6 h with using petroleum ether as a solvent. After evaporating the solvent, the fatty acids were saponified with 2% methanolic NaOH and esterified into methyl esters with 14% BF_3 in methanol. After that fatty acid methyl esters (FAMEs) were analysed by HP Agilent 6890 N Model GC equipped with a FID and SP-2380 FSC. The temperatures of injector and detector were 250ºC and 260ºC, respectively. Temperature of the GC oven was kept at 140ºC for 5 min and then programmed at 240ºC with an accelerating rate of 3ºC/min. The total run time was 41.33 min. For the carrier gas helium was used as the rate of 1 ml/min. By comparing FAME peak relative retention times of sample, identification of fatty acids was carried out. The results were expressed as FID response area % as mean values ± standard deviation in the relative percentages.

ANALYTICAL STANDARDS

The identification of essential oil components was performed by comparing relative retention times to that of authentic samples or by comparison of their relative retention index (RRI) to the n-alkane series (C_9-C_{20}). Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 3 Library) [9, 10] and in-house 'Baser Library of Essential Oil Constituents' built up by genuine compounds and components of known oils, as well as MS literature data [11-13], was used for the identification.
The identification of fatty acids was carried out by comparing FAME peak relative retention times of the sample with the standard 37 components of the FAME mixture (Sigma). Three replicate gas chromatography analyses were performed. Measurements were averaged and results are given as mean ± standard deviation, calculated by analysis of variance using the ANOVA.
As a result, for the identification methods relative retention indices of authentic compounds on the HP Innowax column and/or computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

ANTIOXIDANT ACTIVITY ASSAYS

DPPH

The DPPH free radical scavenging activity measurement was carried out according to the method of L.R. Fukumoto et al. with some modifications [14]. The measurement was carried out on a 96 well microplate. Microdilution series of samples (1 mg/mL, dissolved in HPLC grade MeOH) were made starting with 150 µL. To each well 50 µL of DPPH reagent (100 µM, made with HPLC grade MeOH) was added to gain 200 µL working volume. The microplate was stored at room temperature in dark conditions. The absorbance was measured after 30 minutes at 550 nm using a BMG Labtech FluoStar Optima plate reader. For the blank control HPLC grade MeOH was used instead of the sample. As standard ascorbic acid (0.01 mg/mL, in HPLC grade MeOH) was used. The evaluation of EC_{50} values were carried out with help of GraphPad Prism 6.05. Due to the lack of activity of the hexane extract the EC_{50} value could not be evaluated.

ORAC

The ORAC assay was carried out on a 96-well microplate according to the method of Mielnik et al [15]. 20 µL of extracts of 0.01 mg/mL concentration were mixed with 60 µL of AAPH (12 mM final concentration) and 120 µL of fluorescein solution (70 nM final concentration). For the AAPH and fluorescein solution sodium phosphate buffer, 75 nM, pH=7.4 was used. The fluorescence was measured through 3 hours with 1.5 minutes cycle intervals with FLUOstar Optima BMG Labtech plate-reader. All the experiments were carried out in triplicate, as standard trolox was used. The antioxidant capacity was expressed as µmol Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) Equivalent per mg of dry extract (µmol TE/mg), with help of GraphPad Prism 6.05.

Reducing Power Assay

At the base of the method, which was modified according to Oyaizu, is the reducer matter reducing Fe^{3+} ions to Fe^{2+} ions and absorbance of the complex which is formed after adding $FeCl_3$ is measured [16]. High absorbance means high reducing power. The measurement was done in 700 nm absorbance. For positive control rutin is used.

CUPRAC

According to Apak et al. with some modifications Cu (II) reducing power was analysed [17]. After preparing the mixture of neocouprin and Cu (II) solutions (pH 7), with sample solutions in various concentrations the measurements were done in 450 nm absorbance after waiting 30 minutes at room temperature. For standard Trolox solution in different concentrations was used.

ABTS

For ABTS [2,2'-azinobis (3-ethylbenzotiazolyn-6-sulfonic acid) diammonium salt] assay, ABTS solution was prepared and diluted with ethanol until giving 0.750 absorbance in 734 nm [18]. 0.1 ml extract and 10 µl α-tocopherol were added to 1 ml $ABTS^{+}$ solution and absorbance changing was observed in 734 nm for 6 minutes. For a standard solution α-tocopherol was used. % ABTS rate was calculated as follows:

$$\%\ ABTS\ rate = \frac{[Abs.\ 1 - Abs.\ 2]}{Abs.\ 1} = \times 100$$

RESULTS AND DISCUSSION

Results of antioxidant activities of extracts of *P. pyrrhoblepharus* are shown in Table I. For the antioxidant activities 'DPPH, ORAC, ABTS, CUPRAC and the Reducing Capacity', the ethyl acetate extract of *P. pyrrhoblepharus* is determined as the most active extract in four extracts of the plant with 6.28 ± 0.47 µg/ml (DPPH), 4331.63 ± 49.31 µmol TE/g (ORAC), 0.807 ± 0.012 µg/ml (Reducing Power), 0.164 ± 0.06 mg/ml (CUPRAC) and 50.463% (ABTS) values. The second most active extract is determined as chloroform extract of the plant on five different antioxidant activity assays. It has shown, in

Table I - Results of antioxidant assays

P. pyrrhoblepharus	DPPH (µg/ml)	ORAC (µmol TE/g)	Reducing Power (µg/ml)	CUPRAC (mg/ml)	ABTS (%)
Ethyl acetate	6.28±0.47	4331.63±49.31	0.807±0.012	0.164±0.06	50.463
Chloroform	15.61±1.27	1862.65±41.99	0.789±0.008	0.138±0.03	34.868
Methanol:water	17.71±0.86	1612.13±29.06	0.717±0.013	0.121±0.08	15.992
N-hexane	-	237.14±43.57	0.118±0.014	0.083±0.05	5.728

previous studies, that, in general, ethyl acetate and chloroform extracts have more antioxidant activity than other extracts because they contain more phenolic compounds such as flavonoids, tannins, coumarins, procyanidins. Especially the total ethyl acetate extract is thought to be due to being rich in flavonoid compounds. Many studies have revealed that the antioxidant capacity of extracts is directly proportionate to being rich in flavonoids [19-23].

It is known that compounds capable of fighting free radicals that play an important role in the formation of various diseases (including cancer, aging and DNA damage) have an inhibitory or therapeutic effect on the progression of these diseases [24]. Therefore, the high antioxidant activity in the extracts of our plant is a very important result.

The identified components of the essential oil from *P. pyrrhoblepharus* are listed with relative retention rates and percentages in Table II. GC and GC/MS analyses of the essential oil from *P. pyrrhoblepharus* determined the identification of 59 components representing 84% of the oil. The major components were found with this analysis like hexadecanoic acid (17.1%), spatulenol (10.3%), caryophyllene oxide (9.6%) and heptacosane (5.2%).

The major components of *P. pyrrhoblepharus* were determined as 8,9-dihydroneolongifolene (27%), 9,10-dehydroisolongifolene (10.8%), decahydro-naphthalene (10.6%) and β-caryophyllene (6.5%) in another study [7]. This difference may derive from date and location.

Particularly major component hexadecenoic acid is reported in this genus for the first time. It is interesting to note the presence of β-caryophyllene.

In previous studies major components of essential oils from different *Psephellus* species were shown like this: for *P. huber-morathii* is octanol (17.8%), for *P. hadimensis* is germacrene D (44.3%), for *P. appendicigerus* is β-caryophyllene (17.5%), for *P. pulcherrimus* is germacrene D (17.8%) and for *P. mucroniferus* is germacrene D (29.3%) [25-29].

With the fatty acid analysis, in total 14 fatty acids were identified in the oil of *P. pyrrhoblepharus* as seen in Table III. The amount of saturated fatty acids was found as 31.37% with major fatty acid C16:0 (palmitic acid) (12.86%). The amount of monounsaturated fatty acids (MUFA) were found as 22.76% with major fatty acid C18:1 ω9 (oleic acid) (17.89%). The amount of polyunsaturated fatty acids (PUFA) were found as 17.53% with major fatty acid C18:2 ω6 (linoleic acid) (16.08%). Previous studies about the composition of fatty acids in *Psephellus* and *Centaurea* genus showed that our results are meaningful [30, 31].

Linoleic acid, the major polyunsaturated acid, is necessary, in adequate amounts, to health. A lack of dietary essential fatty acids such as linoleic acid has been implicated in aetiology of diseases including cardiovascular disease and its progression [32, 33]. Also oleic acid, the major monounsaturated fatty acid for the plant, has the capability to lower blood cholesterol levels like linoleic acid [34]. An intake of these fatty acids (oleic and linoleic acids) is promoted

Table II - Composition of essential oil of *P. pyrrhoblepharus*

RRI	Component	%	IM
1360	1-Hexanol	0.2	RRI, MS
1400	Nonanal	0.3	RRI, MS
1497	α-Copaene	0.2	MS
1506	Decanal	0.4	RRI, MS
1553	Linalool	0.1	RRI, MS
1562	Octanol	0.2	RRI, MS
1612	β-Caryophyllene	0.8	RRI, MS
1617	Undecanal	0.3	RRI, MS
1664	Nonanol	0.1	RRI, MS
1687	α-Humulene	0.2	RRI, MS
1719	1-Heptadecene	0.1	MS
1722	Dodecanal	0.2	RRI, MS
1726	Germacrene D	0.2	MS
1740	α-Muurolene	0.1	MS
1766	Decanol	0.2	RRI, MS
1830	Tridecanal	1.4	RRI, MS
1868	(E)-Geranyl acetone	0.3	MS
1898	1,11-Oxydocalamenene	0.1	MS
1941	α-Calacorene	0.3	MS
1945	1,5-Epoxy-salvial-(4)14-ene	1.9	MS
1958	(E)-β-Ionone	0.7	MS
1973	Dodecanol	0.3	RRI, MS
2001	Isocaryophyllene oxide	0.6	MS
2008	Caryophyllene oxide	9.6	RRI, MS
2037	Salvia-4(14)-en-1-one	0.5	MS
2041	Pentadecanal	0.5	RRI, MS
2071	Humulene epoxide-II	1.2	MS
2104	Viridiflorol	0.4	MS
2130	Salviadienol	0.4	MS
2131	Hexahydrofarnesyl acetone	2.5	MS
2144	Spathulenol	10.3	MS
2148	(Z)-3-Hexen-1-yl benzoate	0.1	MS
2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	0.6	MS
2179	nor-Copaonone	0.1	MS
2192	Nonanoic acid	0.6	RRI, MS
2200	3,4-Dimetil-5-pentyl-5H-furan-2-one	0.3	MS
2209	T-Muurolol	0.8	MS
2247	trans-α-Bergamotol	0.4	MS
2255	α-Cadinol	1.0	MS
2267	Guaia-3,9-dien-11-ol	0.4	MS
2278	Torilenol	1.0	MS
2289	4-oxo-α-Ylangene	0.7	MS
2298	Decanoic acid	0.5	RRI, MS
2300	Tricosane	1.8	RRI, MS
2324	Caryophylladienol II	0.8	MS
2369	Eudesma-4(15),7-dien-4β-ol	1.5	MS
2389	Caryophyllenol I	0.6	MS
2392	Caryophyllenol II	1.5	MS
2400	Tetracosane	0.4	RRI, MS
2500	Pentacosane	3.0	RRI, MS
2503	Dodecanoic acid	1.9	RRI, MS
2617	Tridecanoic acid	0.2	RRI, MS
2622	Phytol	3.2	MS

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2670	Tetradecanoic acid	1.9	RRI, MS
2700	Heptacosane	5.2	RRI, MS
2822	Pentadecanoic acid	0.8	RRI, MS
2900	Nonacosane	3.0	RRI, MS
2931	Hexadecanoic acid	17.1	RRI, MS
	TOTAL	84	

Notes: RRI: Relative retention indices calculated against *n*-alkanes; %: calculated from FID data; tr: Trace (< 0.1 %); IM: Identification method based on the relative retention indices (RRI) of authentic compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

Table III - Fatty acids composition of *P. pyrrhoblepharus*

Fatty acids	***P. pyrrhoblepharus***
C 4:0 (Butiric acid)	0.08±0.01[a]
C 6:0 (Caproic acid)	1.25±0.06
C 12:0 (Lauric acid)	1.06±0.04
C 13:0 (Trydecylic acid)	0.78±0.03
C 14:0 (Miristik acid)	2.98±0.06
C 16:0 (Palmitic acid)	12.86±0.26
C 18:0 (Stearic acid)	10.17±0.12
C 21:0 (Heneicosanoic acid)	0.31±0.01
C 22:0 (Behenic acid)	1.23±0.04
C 23:0 (Trycosanoic acid)	0.65±0.02
∑SFA[b]	31.37±0.14
C 18:1 ω9 (Oleic acid)	17.89±0.11
C 20:1 ω9 (Gondoic acid)	4.87±0.06
∑MUFA[b]	22.76±0.13
C 18:2 ω6 (Linoleic acid)	16.08±0.08
C 18:3 ω6 (γ-linolenic acid)	1.45±0.01
∑PUFA[b]	17.53±0.09

Notes: [a] Values reported are means ± SD of 3 lots analyzed.
[b] SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids.

by nutritionists and the health professionals. Oleic acid, with the ability of reducing low-density lipoprotein (LDL) levels and possibly increasing high-density lipoprotein (HDL) levels, is known as a monounsaturated fatty acid in a normal diet [35-37].

4. CONCLUSION

Interest in the use of plant-derived compounds for therapeutic purposes is increasing day by day. So that any approach to treat diseases is extremely valuable. Strong antioxidant activity of *P. pyrrhoblepharus* extracts (especially ethyl acetate and chloroform extracts) may be useful for pharmaceutical and cosmetic industry.
Besides, this is the first report on fatty acids composition of *P. pyrrhoblepharus*. So, it will give a good contribution to the world of science.

Conflicts of Interest: The authors have declared that there are no conflicts of interest.

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