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CHEMICAL COMPOSITION OF ESSENTIAL OIL AND ANTIOXIDANT ACTIVITY OF *Postia puberula*, AN ENDEMIC SPECIES FROM IRAN

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ABSTRACT

Essential oils from the leaves, stems and flowers of *Postia puberula* at the flowering stage were analyzed using GC and GC/MS. The results showed that leaves are abundant of cis-3-hexenyl benzoate (10.75%), benzyl benzoate (8.16%) and caryophyllene oxide (8.12%). The main compounds of stems were benzyl benzoate (21.92%), E-nuciferol (11.58%) and dibutyl phthalate (7.08%), while major components of flowers were benzyl benzoate (9.99%), caryophyllene oxide (8.14%) and E-nuciferol (8.13%). The antioxidant activities of methanol extract were evaluated by DPPH and β-carotene/linoleic acid assays. The results showed that in both methods, leaves had stronger antioxidant activity than other organs.

Key words: benzyl benzoate, caryophyllene oxide, DPPH, β-carotene/linoleic acid, essential oil, *Postia puberula*

INTRODUCTION

Medicinal plants are nature's gift to human beings to help them pursue a disease-free healthy life, and thus they can play an important role in preserving the health. Plants have been used as drugs by humans for thousands of years. Today, all the world's cultures have an extensive knowledge of herbal medicine. Traditional medicine is based on beliefs and practices that existed before development of so-called "modern medicine" or "scientific drug therapy". These practices are part of a country's cultural heritage and are transmitted orally or written [Abad et al. 2012].

Essential oils are mostly natural mixtures of terpenes and terpenoids, most of which are obtained from aromatic and pharmaceutical plants [Yayli et al. 2005]. In addition to their widespread use as flavoring material, essential oils represent a "green" alternative in the nutritional, pharmaceutical, and agricultural fields due to reported antimicrobial, antiviral, nematicidal, antifungal, insecticidal, and antioxidant properties [Chhetri et al. 2015].

Reactive oxygen species (ROS) may cause diseases including cancer, heart diseases, multiple sclerosis, Parkinson's disease, autoimmune diseases, senile dementia malaria, acquired immune deficiency syndrome, stroke, and diabetes [Özgen et al 2004]. Stress, physical damage, viral infection and cytotoxic or carcinogenic compounds, as a consequence of chemical or biological aggression, may cause peroxidation of polyunsaturated fatty acids of cell membranes and liberation of toxic substances such as free radicals. Studies concerning the relationship between the mortality due to cancer and heart diseases and consumption of fruits and vegetables indicated that polyphenols, being present in large amounts in fruits

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and vegetables, have significant decreasing effect on the mortality rate from these diseases [Heim et al. 2002, Hertog et al. 1993, Rice-Evans 2001]. Antioxidant compounds such as phenol and flavonoid, play an important role in preventing chronic diseases by reducing the oxidative damage caused by these highly reactive molecules [Gharibi et al. 2013].

Asteraceae plants are distributed throughout the world and they are most common in the arid and semi-arid regions of subtropical and lower temperate latitudes [Saeidnia et al. 2011]. *Postia* genus belonging to *Asteraceae* family consists of two species that are endemic in Iran. *Postia puberula* is a shrub plant and it's habitat is South of Iran [Mozaffarian 1996].

Chemical composition of essential oil and the antioxidant properties of *P. puberula* extracts have not been previously reported. Therefore, the aim of the present study is the evaluation of essential oil composition and antioxidant properties of it's methanol extracts.

MATERIALS AND METHODS

Sample preparation. *P. puberula* plants (leaves, stems and flowers) were collected at the flowering stage from Khoram Abad located in Lorestan province (Iran) and were dried under shade for a month at room temperature.

Essential oil extraction. The essential oils from leaves, stems and flowers of *P. puberula* plant were extracted by hydro-distillation using Clevenger-type apparatus. Distillation process was performed for 3 h. The obtained essential oils were stored in the freezer at −20°C until analysis.

Analysis of essential oils in different parts of *P. puberula* plant. Flame ionization detector-gas chromatography (FID-GC) was performed using a Hewlett-Packard 6890 with HP-5 capillary column (phenyl methyl siloxane, 25 m × 0.25 mm i.d., 0.25 µm film thickness); carrier gas, He; split ratio, 1 : 25, and flame ionization detector. Temperature program: 60°C (2 min) rising to 240°C at 4°C/min; injector temperature, 250°C; detector temperature, 260°C. GC-MS was performed using Hewlett-Packard 6859 with quadrupole detector, on a HP-5 column (GC), operating at 70 eV ionization energy, using the same temperature program, and carrier gas as mentioned earlier. Retention indices were calculated using retention times of *n*-alkanes that were injected after the oils at the same chromatographic conditions according to Van Den Dool's method 1963.

Identification of the components was done by comparing their mass spectra with those of internal Wiley Gas chromatography-Mass Spectrometry (GC-MS) spectral library, or with published mass spectra and those described by Adams [2001].

Preparation of methanol extracts. The samples (10 g) of leaves, stems and flowers of *P. puberula* plant were extracted separately with 100 ml of 100% methanol for 72 h at room temperature. The extracts were separated from solids by filtering using Whatman No. 1 filter paper. The remaining residue was re-extracted three times and the extracts were pooled. The solvent was removed under vacuum at 45°C, using a rotary vacuum evaporator (IKA RV 06-ML1B 230V, Germany) and the obtained dried leaves, stems and flowers of *P. puberula* plant extracts stored at −4°C until used for further analyses.

DPPH assay. In this method, 50 µL of different concentrations of leaves, stems and flowers of *P. puberula* plant extracts were mixed with methanol solution (1 ml) containing DPPH radicals (0.004%, w/v). After 30 min, the absorbance of specimens was measured at 517 nm using a microplate reader (Bio Tek, U.S.A). The inhibition of free radicals was measured using the following equation:

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

where: A_{blank} is the absorbance of the control reaction (including all reagents, except from defined concentration of a given extract) and A_{sample} is the absorbance of the test. The IC_{50} represents the concentration of extracts that cause 50% inhibition of the radical [Tepe et al. 2006].

β-carotene/linoleic acid assay. In this method, the antioxidant potential of leaves, stems and flowers of *P. puberula* plant extracts was measured by plotting the decolorization of β-carotene/linoleic acid assay. To prepare the β-carotene/linoleic acid solution, 0.5 mg β-carotene was mixed with 1 ml chloroform, and then 25 µL linoleic and 200 mg Tween-40 were added. The chloroform was completely evaporated. In the next stage, 100 ml oxygen-saturated

distilled water was added and the container was vigorously shaken. Then, 2500 µL reaction mixture and 350 µL of the obtained extracts (500 µg/ml) were added to the test tube. In zero time and after 2 h incubation at 50°C, the absorbance of the specimens were measured at 470 nm using a microplate reader (Bio Tek, U.S.A). The antioxidant capacity of the extract was compared with positive tests. All the tests were carried out in triplicate. The activity was expressed as inhibition percentage using the following equation:

$$AA\% = (1 - DR_S / DR_C) \times 100$$

where: AA% is the antioxidant activity, DR_C and DR_S are the degradation rates of β-carotene in the reactant mixture without and with the sample,

$$DR = \ln (a / b) \times 1 / t$$

where: a = initial absorbance at 0 min, b = absorbance at 120 min, and t = 120 [Li and Wang 2009].

Determination of the phenolic compounds. Phenolic compounds were determined according to the method of Amiri [2011] with some modifications. Briefly, 100 µL of the leaves, stems and flowers of *P. puberula* plant extracts (2 mg/ml) were mixed with 1500 µL of Folin-Ciocalteu reagent (diluted tenfold) and 1 ml distilled water was added; after 1 min, 1500 µL of a solution of 20% sodium carbonate was added and the mixture was kept in the dark at room temperature, then absorbance was measured at 760 nm. The same procedure was repeated for all standard gallic acid solutions and the concentration of the phenolic compounds was calculated accordingly and the standard curve was obtained using the following equation:

$$\text{Absorbance: } 0.001 \text{ gallic acid } (\mu g/ml) + 0.111 \ (r^2 = 0.994).$$

Determination of the flavonoid compounds. Here, 500 µL of the leaves, stems and flowers of *P. puberula* plant extracts (2 mg/ml) were mixed with 1500 µL methanol, 100 µL of 10% aluminum trichloride, 100 µL of potassium acetate 1 M, and 2.8 mL of distilled water. After 10 min at room temperature, the absorbance was determined at 420 nm. The same procedure was repeated for all standard quercetin solutions and the concentration of flavonoid compounds was calculated accordingly and the standard curve was obtained using the following equation [Karamian et al. 2014]:

$$\text{Absorbance: } 0.0091 \text{ quercetin } (\mu g/ml) + 0.0206 \ (r^2 = 0.995).$$

Statistical analysis. Experimental results were represented as mean ± standard error (SE) of three parallel measurements and analyzed by the Minitab software. Differences between means were determined using Tukey's comparisons.

RESULTS

Essential oils. Essential oils analysis of the leaves, stems and flowers of *P. puberula* plant results are shown in Table 1. Results showed that leaves are abundant of cis-3-hexenyl benzoate (10.75%), benzyl benzoate (8.16%) and caryophyllene oxide (8.12%). The main compounds of the stems were benzyl benzoate (21.92%), E-nuciferol (11.58%) and dibutyl phthalate (7.08%) and the major constituents of the flowers were benzyl benzoate (9.99%), caryophyllene oxide (8.14%) and E-nuciferol (8.13%). The compound of essential oils are divided to terpenoids, aliphatic and aromatic esters. In leaves and flowers, terpenoids have the highest percentage, while aliphatic compounds identified as a main group in stems.

DPPH assay. Antioxidant activity (IC_{50}) of leaves, stems and flowers methanolic extract compared to BHT are shown in Figure 1 and Table 2. The results show that the antioxidant activity of BHT is higher than all plant different parts. Among different parts, the leaves have stronger antioxidant activity. The antioxidant capacity of different parts and BHT is as follows (BHT > leaves > flowers > > stems).

β-carotene/linoleic acid assay. In this assay, the antioxidant activity of BHT, leaves, stems and flowers methanolic extract were 92.71%, 64.62%, 51.91% and 43.95%, respectively. Results show that the BHT has higher antioxidant activity compared to plant organs. Between different organs, leaves have higher antioxidant activity (BHT > leaves > stems > flowers). All data in this method were statistically significant (Fig. 2 and Tab. 2).

Table 1. Essential oil composition of leaves, stems and flowers of *Postia puberula*

Compounds name	RI	Leaves	Stems	Flowers
1	2	3	4	5
Hexanal	800	7.68	1.53	0.41
E-3-Hexen-1-ol	844	–	1.77	–
E-2-Hexanal	854	–	–	0.29
n-Hexanol	867	–	–	0.20
α-Pinene	939	–	–	0.16
n-Decane	999	–	0.48	–
α-Terpinene	1018	–	–	0.54
Para Cymene	1026	–	–	0.12
γ-Terpinene	1062	–	–	1.43
Octanol	1070	–	–	0.16
Terpinolene	1088	–	–	0.44
Linalool	1098	0.71	–	1.81
Nonanal	1102	0.58	–	–
n-Nonanal	1102	–	0.58	0.18
α-Campholenal	1122	0.97	–	–
Cis verbenol	1137	–	–	1.52
Cis-beta-Terpineol	1144	–	–	0.60
Isopulegol	1156	1.39	–	–
p-Mentha-1,5-dien-8-ol	1166	–	–	0.74
Octanoic acid	1167	–	–	0.36
Terpinen-4-ol	1174	–	–	7.38
Myrtenol	1194	–	–	7.67
n-Dodecane	1199	0.52	0.31	–
n-Decanal	1204	0.95	0.91	–
Verbenone	1204	–	–	0.15
Trans pipertiol	1205	–	–	0.21
Trans pipertion	1207	–	–	0.48
Nerol	1228	–	0.66	0.37
Geraniol	1255	–	–	0.25
n-Decanal	1272	–	–	0.39
Carvacrol	1298	–	–	0.44
n-Teridecane	1299	1.80	0.27	0.34
Myrtenyl acetate	1324	–	–	1.46
Eugenol	1356	0.65	–	–
E-2-undecenal	1357	–	0.31	–
n-Tetradecane	1400	0.88	0.47	–
6,10-dimethyl-2-undecanone	1404	1.28	5.69	0.87
Trans-alpha-Bergamotene	1436	–	0.27	6.73
Aromadendrene	1439	4.98	–	–
Geranyl acetone	1453	0.52	–	–

1	2	3	4	5
Neryl acetone	1453	–	0.27	–
α-Humulene	1454	–	–	0.52
β-Lonone	1485	0.97	–	–
Neryl isobutyrate	1491	0.48	4.09	0.30
α-Selinene	1494	0.48		–
n-Pentadecane	1500	–	0.31	–
α-Bulnesene	1505	–	–	0.28
Hedycayol	1546	–	–	0.47
E-Nerolidol	1564	0.96	0.54	1.27
Cis-3-Hexenyl benzoate	1565	10.75	1.02	6.01
Dodecanoic acid	1568	–	0.31	1.05
Hexyl benzoate	1579	2.20	1.03	3.44
Caryophyllene oxide	1582	8.12	0.62	8.14
Viridiflorol	1590	0.96	–	–
n-Hexadecane	1600	1.39	1.04	0.35
t-Cadinol	1638	–	0.40	3.24
δ-Cadinol	1647	1.61	3.28	–
Eudesmol	1649	–	6.07	4.13
Tau-cadinol	1650	7.86	–	–
α-Cadinol	1669	5.41	–	–
E-Asaron	1675	–	0.37	–
Eudesm-7(11)-en-4-ol	1700	–	–	0.13
E-Nuciferol	1758	3.24	11.58	8.13
Benzyl benzoate	1759	8.16	21.92	9.99
n-Octadecane	1800	0.91	0.76	–
Henadecanic acid	1959	1.16	1.87	–
Dibutyl phthalate	2085	–	7.08	–
Phytol	2114	1.03	6.66	–
Terpenoids		38.45	24.06	56.88
Monoterpene hydrocarbons		–	–	2.69
Oxygenated monoterpenes		4.3	0.66	21.62
Sesquiterpene hydrocarbons		32.67	22.32	16.44
Oxygenated sesquiterpene		1.48	1.08	16.13
Aliphatics		29.87	27.25	12.54
Alcohols		1.03	8.45	0.83
Aldehydes		10.43	3.33	1.27
Alkanes		3.7	3.64	0.69
Ketone		–	5.69	0.87
Esters		13.43	6.14	9.75
Aromatic esters		8.16	29	9.99
Other		2.24	2.18	2.87
Total		78.6	82.49	83.15

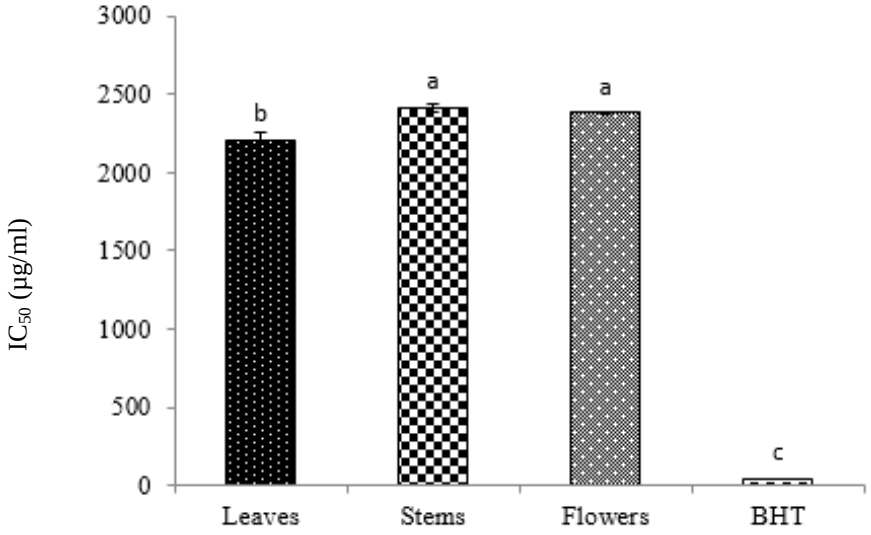

Fig. 1. Free radical scavenging of leaves, stems and flowers of *Postia puberula* compared with BHT by DPPH assays. The same letter(s) are not significantly different at p 0.05 probability (n = 3)

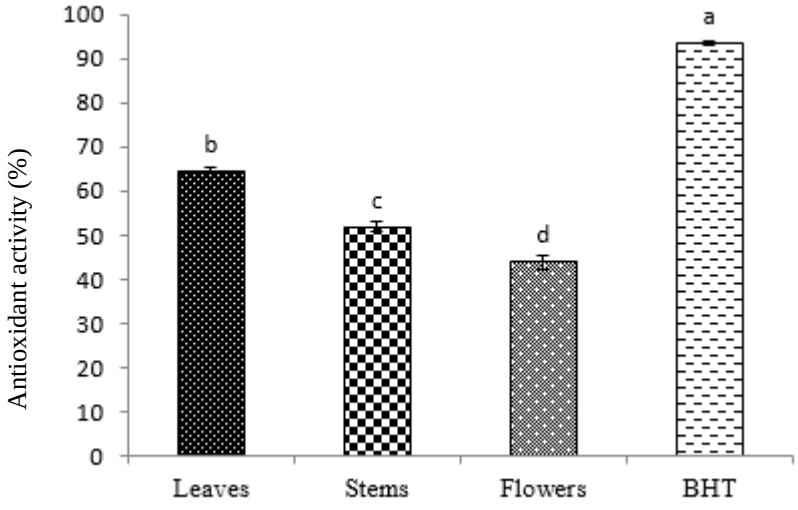

Fig. 2. Antioxidant activity of leaves, stems and flowers of *Postia puberula* compared with BHT by β-carotene-linoleic acid assay. All data in this method were statistically significant ± standard error (n = 3; $p < 0.05$)

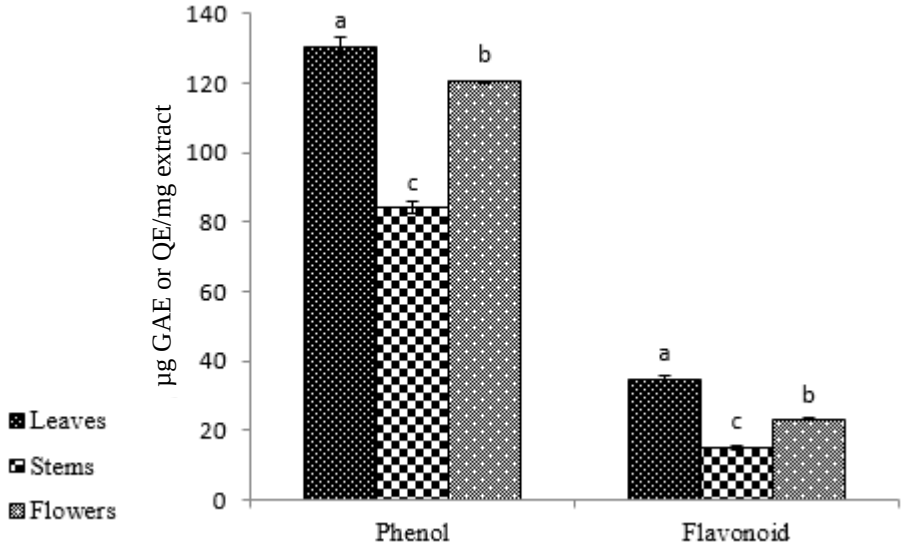

Fig. 3. Phenol and flavonoid contents of leaves, stems and flowers of *Postia puberula*. The leaves has more phenol and flavonoids in comparison to the stems and flowers. Data are statistically significant ± standard error (n = 3; $p < 0.05$)

Table 2. Antioxidant activity, phenol and flavonoid content of leaves, stems and flowers of *Postia puberula*

	Antioxidant activity		Phenol (µg gallic acid/ mg extract)	Flavonoid (µg quercetin/ mg extract)
	DPPH (IC_{50}(µg/ml))	β-carotene linoleic acid		
Leaves	2204.28 ±51.40 [b]	64.62 ±0.72 [b]	130.5 ±2.60 [a]	34.59 ±1.32 [a]
Stems	2411.54 ±23.64 [a]	51.91 ±1.00 [c]	84 ±1.73 [c]	14.99 ±0.44 [c]
Flowers	2380.56 ±10.87 [a]	43.95 ±1.47 [d]	120.5 ±0.29 [b]	24.37 ±0.27 [b]
BHT	42.33 ±0.91 [c]	92.71 ±0.43 [a]		

Same letter indices are not statistically significant ± standard error (n = 3; $p > 0.05$)

Phenol and flavonoid content. The amount of phenol and flavonoid of leaves, stems and flowers methanolic extracts are shown in Figure 3 and Table 2. Results shown that the content of phenol and flavonoid in leaves is more than that from stems and flowers, also flowers have more phenol and flavonoid compared to stems. All data were statistically significant.

DISCUSSION

In this study, the roles of different organs on essential oils composition, antioxidant activity and phenol and flavonoid content of *Postia puberula* different parts, were investigated.

Results showed that the main components of essential oils were different in various parts. Kahriman

et al. [2011] showed that composition of essential oil in different organs of *Senecio pandurifolius* are different, and that α-cuprenene and γ-curcumene were the main compounds in stem and flower, respectively. Also α-zingibereneas, one of the main compounds in oil of leaf and stem, was not found in flower.

Caryophyllene oxide as one of the main constituents in essential oil of *P. puberula* is used as preservative in food, drugs and cosmetics, and as an antimicrobial and antifungal agent [Yang et al. 2000]. Also it is a main compound in some species of *Asteraceae* family such as *Centaurea sessilis* [Yayli et al. 2005], *C. reuterana* var. *reuterana* [Karamenderes et al. 2008], *C. pseudoscabiosa* subsp. *Pseudoscabiosa* [Flamini et al. 2002] and *Artemisia campestris* subsp. *Campestrs* [Judzentieneet et al. 2010]. Benzyl benzoate as one of the main constituent of stems, leaves and flowers of *P. puberula,* is present in some species of *Asteraceae* family such as *Helichrysum oocephalum* Boiss [Firouznia et al. 2007]. Also, in essential oil of several species of *Cinnamomum,* benzyl benzoate was one of the major components. Benzyl benzoate is used as a fragrance ingredient, artificial flavor, preservative, and solvent. It is also considered an over the counter drug and can be used for scabies or lice treatment. It is an ester of benzyl alcohol and benzoic acid [Sallah et al. 2016].

Arituluk et al. found that IC_{50} values of *Tanacetum. Armenum*, *T. cadmeum* subsp. *Cadmium*, *T. cilicicum, T. praeteritum* subsp. *massicyticum* and *T. praeteritum* subsp. *Praeteritum* were 247.57, 236.58, 249.17, 197.82 and 197.82 (μg/mL), respectively. In addition, phenol and flavonoid contents of these species were 58.86 mg GAE/g extract, 7.69 mg QE/g extract, 131.24 mg GAE/g extract, 23.83 mg QE/g extract, 33.14 mg GAE/g extract, 33.14 mg QE/g extract, 149.93 mg GAE/g extract, 33.42 mg QE/g extract and 112.67 mg GAE/g extract, 10.71 mg QE/g extract, respectively.

The antioxidant activity (IC_{50} value) of *Achillea pachycephalla, A. kellalensis, A. aucheri* were previously reported as 240, 518, 844 (μg/ml), also their phenol of these species were (60.65, 30.07 and 15.55 mg tannic acid /g DM) respectively [Gharibi et al. 2013].

According to report of Parejo et al. [2005], the IC_{50} values of crude extract, defatted extract, hexane, ethyl acetate and aqueous of *Tagetes maxima* were determined as 27.17 ±1.69, 13.11 ±1.11, 46.63 ±1.16, 14.45 ±0.27 and 19.08 ±0.85 and their phenol contents were 188.02 ±13.01, 272.64 ±24.38, 123.03 ±11.36, 392.58 ±20.01 and 199.54 ±14.48 equivalents of GAE/mg dry extract, respectively.

As it can be seen from our results given above, the plant extracts with more phenol content have a stronger antioxidant activity by DPPH assay.

The high antioxidant activity of leaves can be attributed to the higher content of phenol and flavonoid. In DPPH test, following the leaves, the antioxidant activity of the flower is more than that of the stem, while in β-carotene-linoleic acid method, stems have stronger antioxidant capacity. High antioxidant activity of stems in β-carotene-linoleic acid can be related to other compound, except from phenol and flavonoid, such as carotenoids and saponins or other non-polar compounds. Due to the lack of data on the percentage inhibition of linoleic acid oxidation of *P. puberula*, we could not compare the results of our present study with the literature references. However, the percentage inhibitions of methanol extracts determined in our study are in close agreement with some *Asteraceae* family such as the findings of Djidel and Khennouf [2014], who reported the linoleic acid peroxidation for *Artemisia campestris* L. as being 79%. Also in this method, the antioxidant activity of some species of *Centaurea* such as *Centaurea ensiformis*, *C. pulcherrima* var. *pulcherrima* and *C. mucronifera* were 85.1%, 73.8% and 35.2%, respectively [Tepe et al. 2006, Ugur et al. 2009, Aktumsek et al. 2013].

CONCLUSIONS

Considering the traditional uses of *Postia puberula* in foods and traditional medicine, and according to the results of this study, indicating that the antioxidant activity of leaves is higher than other organs, it is suggested that in the future the use of this plant leaves are very important.

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