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Full Length Research Paper

Screening of chemical composition of essential oil, mineral elements and antioxidant activity in *Pulicaria Undulata* (L.) C. A. Mey from Iran

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Pulicaria undulata **(L.) C.A. Mey is a medicinal plant used to treat inflammation. It is also an insect repellent and herbal tea. In this study, concentration of macro minerals (that is, Ca, K and Mg) and micro minerals (major and trace elements) (that is, Fe, Cu, Na and Mn) and P, were estimated in *Pulicaria undulata* from the Sistan and Baluchestan province. Sample preparation using microwave digestion was used for mineralization of this plant. Atomic absorption spectrophotometry (AAS) and UV-Visible spectrophotometry were used to quantify metal levels and Phosphorus, respectively. Essential oil from aerial parts of *P. undulata* was obtained by hydrodistillation. The chemical constituents of essential oil of aerial parts were analyzed by GC-FID and GC/MS techniques. The main components identified in the oil were 4-Terpineole (20.12%), alpha terpinene (4.02%), gamma terpinene (7.00%), cis-sabinene hydrate (8.29%), Linalool (5.60%), 1S-cis-calamenene (13.37%) and Junipene (8.66%). In addition, antioxidant activity of ethanolic extract was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method.**

Key words: Macro and micro minerals, microwave digestion, *Pulicaria undulata*, antioxidant activity.

INTRODUCTION

Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. In nature essential oils play an important role in the protection of the plants as antibactrials, antivirals, antifungals and insecticides. They are usually obtained by steam or hydro- distillation. Known for their antiseptic, that is, bactericidal, virucidal, fungicidal, medicinal properties and their fragrance (Bakkali et al., 2008). There is our day, an increasing interest in the measurement and use of plant antioxidants for scientific research as well as industrial purposes. This is mainly due to their strong biological activity, exceeding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis (Suhaj, 2006). Therefore, the need exits for safe, economic, powerful and natural antioxidants to replace these synthetic ones (Tadhani et al., 2007). Scientists could qualitatively detect small amounts of several minerals elements in living organisms. The trace elements found in living organisms may be essential, that is, indispensable for growth and health or they may be nonessential, fortuitious reminders of our geochemical origins or indicators of environmental exposure (Ozcan, 2004). Human as well as animal, studies originally showed that optimal intakes of elements such as calcium, sodium, potassium, manganese, magnesium and copper could reduce individual risk factors including those related to cardiovascular disease (Mertz, 1982; Sanchez-Castillo et al., 1998). *Pulicaria undulate* (L.) C. A. Mey.

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[syn. *Pulicaria crispa* (forssk.) *Olive.Francoeuria crispa* (forssk.) Cass] is an annual herb or sometimes a perennial sub shrub belonging to the family Asteraceae producing small bright yellow flowers. This species is distributed in Iran, Saudi Arabia, Kuwait, Iraq, Egypt, Afghanistan, Pakistan, India and parts of North and West tropical Africa (Boulos, 2002; Al-Rawi, 1987).

Genus Pulicaria (family Compositae, tribe Inuleae) is represented in the flora of Iran by five species (Nematollahi et al., 2006). *P. undulata* is a medicinal plant used by people of Southern Egypt and Saudi Arabia to treat inflammation. It is also as an insect repellent (Stavri et al., 2008) and is also used as a herbal tea (Ross et al., 1997). The composition of steam distilled oil of the fresh arial parts of *P. undulata* (L.) kastel (from Saudi Arabia) was investigated by GC/MS. The oil is rich in phenolic compounds and monoterpene hydrocarbons and comparatively low in sesquiterpene hydrocarbons (Mossa et al., 1987). The essential oil of *P. undulata* aerial parts exhibited activity against Gram-positive and Gram-negative bacteria (EL-Kamali et al., 1998). In this study for first time concentration of macro minerals (that is, Ca, K and Mg) and micro minerals (major and trace elements) (that is, Fe, Cu, Na and Mn) and P, were estimated in *P. undulata* of Saravan area in Sistan and Baluchestan province, Then the essential oil was also obtained from aerial parts of *P. undulata* by hydrodistillation. The chemical constituents of essential oil of aerial parts were analyzed by GC-FID and GC/MS techniques and many components were identified in the oil and also the antioxidant activity of ethanolic extract was evaluated by (DPPH) radical scavenging method.

MATERIALS AND METHODS

Plant materials

P. undulata was collected in November, 2009 from Saravan area of Sistan and Baluchestan province in Iran. The taxonomic idendification of plant materials was confirmed by Valizadeh in the Department of biology in University of Sistan and Baluchestan. The collected plant materials were dried in the shade and aerial parts were separated from the root. The voucher specimen has been deposited at the herbarium of the Department of Biology at the University of Sistan and Baluchestan.

Preparation of ethanolic extract

Ten gramme of arial part of *P. undulata* was finely ground using a homogeniser and was extracted with ethanol at room temperature for 24 h. This mixture was then filtered using whatman No. 42 filter paper to remove the debris and extract was then evaporated at 40°C using a rotary evaporator.

Isolation of the essential oil

The finely dry powdered aerial parts (40 g) were subjected for 2 h to hydrodistillation using a Clevenger-type apparatus. The obtained essential oil was collected, and dried over anhydrous sodium sulphate and kept at 4°C until analysis.

GC-MS analysis

The analysis of the essential oil was performed using an Agilent 6890 GC, equipped with a HP-5MS capillary column (30 m × 0.25 mm i.d. 0.25 µm) and an Agilent 5973 Mass selective detector. For GC-MS detection an EI ionization system with ionization energy of 70 eV was used. Helium was used as the carrier gas, at a flow rate of 0.8 ml/min. Injector temperature was set at 290°C, column temperature was initially kept at 50°C for 5 min, then gradually increased to 240°C at a rate of 4°C /min; and finally raised to 300°C at 15°C /min and was kept for 3 min.

The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on HP-5MS capillary column, and by matching of their mass spectra of peaks with those obtained from authentic samples and/ or the wiley NIST 7 library spectra and published data (Adams, 2007).

Analysis of inorganic elements in Pulicaria undulate

Microwave digestion

0.5 g of crushed air-dried aerial parts of *P. undulata* was mixed with a 10 ml concentrated HNO_3 in the beaker glass was placed inside a domestic Microwave oven. Sample was irradiated at a 900 W power for 10 min. Then, a 5 ml of concentrated HCl was added and irradiation was continued for another 5 min. After digestion, the vessel was cooled, filtered (Whatman No. 42 filter paper) and diluted with double distilled water to a final volume of 100 ml. Solution was used for elemental analysis by atomic absorption spectrophotometer model Philips PU 9100X and UV- Visible spectrophotometry were used to quantify metal levels and Phosphorus quantity, respectively. Atomic absorption spectrophotometer was used for determination of Ca, Mg, K, Fe, Cr and Mn, the phosphor was estimated by UV- Visible spectrophotometer.

Determination of phosphor

Standard solutions of NaH_2PO_4 salt at concentrations of 4,5,6 ppm was produced. The amount of phosphorus in the sample was represented as Molybdat-Vanadat standard determined from its standard curve observed with a UV-Visible spectrophotometer at a wavelength of 420 nm.

DPPH free radical-scavenging assay

The antioxidant activities of the extracts were assessed on the

basis of the radical scavenging effect of the stable DPPH free radical, and the assay was carried out as described by (Brand et al., 1995).

The DPPH solution showed a maximum absorption band at 517 nm and was intensely colored in violet. The absorption and color intensity decreased when DPPH was reduced by an antioxidant compound. The remaining DPPH radical corresponded inversely to the radical scavenging activity of the antioxidant. Evaluating the scavenging activity of ethanolic extracts, its aliquots (15, 30, 60 and 90 µg) were dissolved in 1 mL MeOH, and were added to 1 mL DPPH (0.1 mM) solution at room temperature. The absorbance at 517 nm was measured at 20 min against a blank (1 mL MeOH in 1 mL DPPH solution) using a spectrophotometer. The results are expressed as percentage of reduction of the initial DPPH radical absorption by the test samples. The DPPH radical scavenging activity were compared with those obtained from BHT standard. Inhibition free radical DPPH percentage (I%) was calculated as follows :

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

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the linear regression algorithm of the graph, which was plotted as inhibition percentage against extract concentration. For the calculation of these values, microsoft excel software was used. All analyses were performed in triplicate.

RESULTS AND DISCUSSION

Chemical compostion of the essential oil

The yield of volatile oil of *Pulicaria undulata* obtained by hydrodistillation of the finely powdered aerial parts was 0.5% (v/w).

The oil was light yellow and with a perfumery odor. The chemical composition of the oil is presented in Table 1. The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on HP-5 MS capillary column, and by matching of their mass spectra of peaks with those obtained from authentic samples and/ or the wiley NIST 7 library spectra and published data (Adams, 2007). The GC/MS chromatogram of the oil revealed the presence of monoterpenes (14.51%), oxygenataed monoterpenes (54.41%), sesquiterpenes (29.20%), oxygenataed sesquiterpenes (1.14%) and (1.41%) as other compounds.

The main monoterpene component was 4-terpineole (20.12%), alpha-terpinene (4.02%), gamma-terpinene (7.00%), cis-sabinene hydrate (8.29%), linalool (5.60%). The main sesquiterpene component was j unipene (8.66%) and 1S-Cis-calamenene (13.37%). The high content of oxygenated compounds might explain the characteristic and fragrant odour of the oil. Our results of some components of essential oil of P. undulata show minor differences when compared with literature (EL-Kamali et al., 2009).

This differences might be due to growth conditions, genetic factors, geographical variations and analytical procedures.

Mineral element

In this work for first time, sample preparation with microwave digestion was used for mineralization of this plant. Atomic absorption spectrophotometer was used for determination of Ca, Mg, K, Fe, Cr and Mn. Phosphor was determined by UV- Visible spectrophotometer. Table 2 shows the percent of various metals in this plant. According to results, the highest mineral contents were Ca and K. This work attempts to contribute to knowledge of the nutritional properties of this plant and the rapid sample preparation using a microwave oven was significantly in favor in digestion method.

Antioxidant activity

The scavenging effect of ethanolic extract of *P. undulata* under investigation on DPPH radicals are shown in Figure 1. The reduction ability DPPH radicals formation was determined by the decrease in its absobance at 517 nm induced by antioxidants.

The effect of antioxidants on DPPH radical scavenging is though to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). According to antioxidant outcomes, the amount of IC_{50} ethanolic extracts and BHT were 63.47 and 25.86 ppm respectively. Ultimetly, highlited that antioxidant activity of ethanolic extracts is weaker than BHT.

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Table 1. Chemical composition of the essential oil of arial parts of *P. undulate.*

NO	Components	RT (Min)	(%)
1	α-thujene	10.37	0.14
2	α-pinene	10.68	0.52
3	Sabinene	12.61	0.21
4	β-myrcene	13.56	0.07
5	1-phellandrene	14.16	0.08
6	α-terpinene	14.96	4.02
7	1,8-Cineole	15.55	1.00
8	γ-terpinene	17.25	7.00
9	Trans-sabinene hydrate	17.65	2.71
10	α-terpinolene	18.58	2.14
11	Cis-sabinene hydrate	19.41	8.29
12	1,3,8-para-menthatriene	19.68	0.22
13	Isolimonenol	21.34	2.37
14	4-terpineole	23.71	20.12
15	α-terpineol	24.08	2.45
16	Myrtenol	24.50	5.77
17	z-citral	26.19	1.00
18	Linalool	27.25	5.60
19	E-citral	27.59	1.32
20	Thymol	28.70	0.48
21	α-fenchene	28.95	0.11
22	Myrtenyl acetate	29.90	0.94
23	Copaene	32.04	0.09
24	Geranyl acetate	32.35	0.15
25	Trans-caryophyllene	33.88	0.13
26	α-amorphene	36.26	0.13
27	Δ-gurjunene	36.82	0.05
28	α-muurolene	37.24	0.26
29	γ -cadinene	37.80	0.29
30	Δ-cadinene	38.21	1.03
31	Trans-gamma-bisabolene	38.48	0.61
32	Citronellyl valerate	40.22	0.29
33	Caryophyllene oxide	40.55	0.62
34	Fenenol	42.37	0.30
35	Junipene	43.01	8.66
36	Trans-calamenene	43.55	2.66
37	Valerenol	43.91	0.23
38	Cadalene	44.05	1.92
39	1S-cis-calamenene	45.12	13.37
40	Neryl acetate	59.09	0.32

Table 2. Percentages of mineral elements in *P. undulata*

No	Elements	(%)
1	Potassium	1.969
2	Calcium	3.241
3	Magnesium	0.426
4	Iron	0.366
5	Manganese	0.152
6	Sodium	0.209
7	Copper	0.010
8	chromium	0.015
9	phosphor	0.204

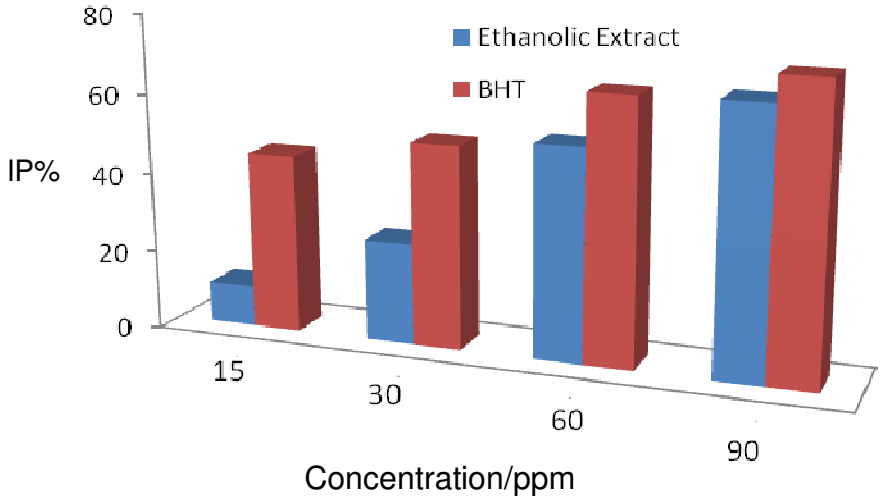

Figure 1. Comparison of scavenging effect of BHT and ethanolic extracts on DPPH radicals. *IP: Inhibitory percent.

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