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ORIGINAL ARTICLE

Antioxydant activities from the aerial parts of *Pulicaria jaubertii*

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ABSTRACT

In order to evaluate the potential of medicinal effects of the popular edible *Pulicaria jaubertii* in Yemen, the chloroform ($CHCl_3$), ethylacetate (AcOEt) and *n*-butanol (*n*-BuOH) extracts from leaves were investigated using a model system. AcOEt extract which had high level of polyphenols and flavonoids, exhibited the most potent antioxidant capacity in each assay, showing a high dose dependant manner scavenging radical activity (DPPH°), lipid peroxidation inhibition (Fe^{2+}/Ascorbate system), OH radical quenching (Deoxyribose assay), ferrous chelating capacity and a moderate reducing power (Fe^{3+}-Fe^{2+}) compared to the *n*-BuOH extract which proved a moderate effect, and to $CHCl_3$ which was the weakest extract. This study suggested that the differences of the potency of the antioxidant activity may be explained by the differences in the polyphenol and flavonoid levels.

Key words: *Pulicaria jaubertii*, Polyphenols, flavonoids, antioxidant activity.

Introduction

The genus *Pulicaria* which belongs to the Asteraceae family (Compositae, trible Inuleae, subtrible Inulinae), comprises more than 77 species widespread all around the world (Dubaie and El-Khulaidi, 1993; Anderberg 1991). Chemically, this genus is not homogenous. As pointed out previously some species contain monoterpenes, diterpenes, sesquiterpene lactones (Abdel-Mogib *et al* 1990; Dendougui *et al* 2000). and caryophyllane derivatives (Hafez *et al*., 1987). Also the literature reports that *Pulicaria* species afforded different flavonoid profiles (Rizk *et al.,1993*). The *Pulicaria* species proved various activities such as anti-inflammatory, antilukemic (Al-Yahaya *et al*.,1984). potential cancer chemopreventive and cytotoxic agents (Al-Yahaya *et al*.,1988).The *Pulicaria jaubertii* indigenous to Yemen, locally known as Anssif, is traditionally used as diuretic, pyritic conditions in urogenetic organs, and to cure fever. The flowers of *Pulicaria jaubertii* was also used as spice and to make various delicious foods. Some investigation reported that this species reveal antimicrobial, antifungal, antimalaria and insecticides properties (Dubaie and El-Khulaidi., 2005).

Free radicals are generated by a process known as redox cycling and they are catalysed by transition metals, to cause DNA and RNA damage, thiol oxidation and lipid peroxidation (Halliwell and Guttridge., 1999; Halliwell 1994). The great potential of free radicals to react with various compounds by electron transfer, proton transfer, H-atom abstraction or addition reaction may involved in the pathological of various diseases (Halliwell.,1993; . Havsteen., 1983).Many plant compounds can scavenge reactive oxygen species (ROS) and

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thereby directly reduce-oxidative stress (Walgren *et al*.,2000b).Among these, flavonoids seem to be potent candidates because they show broad pharmacological activities and widely distributed in many edible plants (Rice-Evans *et al*.,1996).The beneficial effect of flavonoids is mainly associated with the different various antioxidative mechanisms which act as enzyme inhibitor, reducing agents, trapping free radical and by acting as iron-chelating (Bravo.,1998;Hollman.,2001).

In this respect, since up to the present, there is no study on the chemical or the antioxidative activity of *Pulicaria jaubertii*, the aim of this study was to investigate the antioxidative activity of different extracts of the leaves of *Pulicaria jaubertii*: $CHCl_3$, AcOEt and *n*-BuOH extracts as new potential source of natural antioxidants.

Materials and methods

Plant material

The leaves of *Pulicaria jaubertii* were collected from Aljar region (Hajja - Yemen) in October 2008. The plant was taxonomically identified by Pr. Abdellah Amine (Sana'a University). A voucher specimen of the plant material has been deposited at the department of biology (Sana'a University).

Preparation of the extracts

Dried and powdered leaves (400 g) of *P. jaubertii* were extracted with 70% MeOH solution three times during 24 hours. The aqueous MeOH extract was concentrated to dryness; the residue was dissolved in water (200 ml). The resulting solution was extracted successively with petroleum ether, $CHCl_3$, AcOEt and *n*-BuOH. The organic layers were dried with Na_2SO_4 to give after concentration the extracts $CHCl_3$ (2 g), AcOEt (2.5 g) and *n*-BuOH (6 g) respectively.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH°), potassium ferricyanide, gallic acid, ethylenediamine tetra acetic acid (EDTA), ferrozine, Folin- Ciocalteus's phenol reagent, quercetin, ascorbic acid, ferric chloride and sodium carbonate were from sigma, sigma Aldrich. All the chemicals used including the solvents, were of analytical grade.

Determination of antioxidant activity

Determination of DPPH radical scavenging activity

The ability to scavenge the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH°) was determined based on the method of Ohinishi *et al*(Ohinishi *et al.*,1994).with minor modifications. A solution of 0.2 mM DPPH in methanol was prepared and 1 ml of this solution was mixed with 1 ml of extract in methanol (5 to 150 µg/ml).The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. A control sample containing the same volume of solvent in place of extract was used to measure the maximum DPPH absorbance. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid and quercetin were used as references. Results were expressed as percentage of inhibition of the DPPH radical according to the following equation:

$$\% \text{ Inhibition of DPPH} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Determination of reducing power

The reducing power of *P. jaubertii* leaves extracts was determined according to Oyaizu (Oyaizu.,1986). Different amounts of the extracts were suspended in distilled water and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% $K_3Fe(CN)_6$. The mixture was incubated at 50 °C for 20 min, 2.5 ml of 10% TCA was added to the mixture and centrifuged at 3000 rpm for 10 min. 2.5 ml of upper layer of the solution was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% $FeCl_3$, and the absorbance was measured at 700 nm. Increase in absorbance of reaction mixture indicated reducing power. Ascorbic acid and quercetin were used as references.

Inhibition of lipid peroxide formation induced by Fe^{2+}/ascorbic acid system

The reaction mixture containing rat liver homogenate (0.1 ml, 25% w/v) in Tris-HCl (30 mM), ferrous ammonium sulfate (0.16 mM), ascorbic acid (0.06 mM) and different concentrations of the extract (from 50 to 600 µg/ml) in a final volume of 0.5 ml was incubated for 1h at 37°C and the resulting thiobarbituric reacting substance (TBARS) was measured. A 0.4 ml aliquot of the reaction mixture was treated with sodium dodecyl sulfate (0.2 ml, 8.1%), thiobarbituric acid (1.5 ml, 0.8%), and acetic acid (1.5 ml, 20%, pH 3.5), made to a total volume of 4 ml by adding distilled water, and kept in a water bath at 95°C for 1 h. After cooling, 4 ml of *n*-BuOH was added. After shaking and centrifuging, the organic layer was separated and the absorbance measured at 532 nm (Ohkawa *et al*.,1979).Ascorbic acid and quercetin were used as references.

Determination of OH° scavenging

Scavenging of OH° was determined by the method of Halliwell *et al*(. Halliwell *et al.*,1987). OH° were generated by incubating the following reagents in a final volume of 1.2 ml 10 mM KH_2PO_4-KOH buffer (pH 7.4) at 37 °C for 60 min: 1.4 mM H_2O_2, 100 µM $FeCl_3$ and 2.8 mM deoxyribose, 100 µM EDTA, and 100 µM ascorbic acid in presence or absence (control) of the extract. Ascorbic acid was added in the end to start the reaction. Degradations of deoxyribose sugar induced by OH° was determined by addition of 1 ml TBA (1% w/v) and 1 ml TCA (5.0% w/v) and heating at 100 °C for 20 min. The pink chromogen formed was determined by measuring its absorbance at 535 nm. Quercetin was used as reference.

Ferrous-chelating ability

The chelating of ferrous ions by *P. jaubertii* extract was estimated by the method of Dinis *et al.* (2006). Briefly, 50 µl of 2 mM $FeCl_2$ was added to the extract (1 ml). The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance was measured spectrophotometrically at 562 nm. EDTA was used as reference.

Determination of total phenolic contents

Total phenolic content was determined using Folin-Ciocalteu reagent as adapted from Singleton and Rossi (Oyaizu, 1986), with slight modifications. 100 µl of extract was mixed with 250 µl of Folin-Ciocalteu reagent (1N) and allowed to stand at room temperature for 2 min. 1250 µl of sodium carbonate (20%) was added, and the mixture was mixed and allowed to stand at room temperature in the dark for 2 h. The absorbance was read at 765 nm, and the total polyphenols concentration was calculated from a calibration curve, using gallic acid as standard (50–1000 mg/L). The results were expressed as gallic acid equivalents (GAE)/g extract.

Determination of flavonoids

Total flavonoid content was determined using the method of Ordon Ez *et al.* (Ordon Ez *et al* .,2006). A volume of 0.5 ml of 2 % $AlCl_3$ ethanol solution was added to 0.5 ml of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoids were calculated as quercetin (mg/g) using the calibration curve. Results were expressed as mg quercetin equivalents (QE) / g extract.

Statistical analysis

All assays were carried in triplicates and results expressed as means ± standard deviation. IC_{50}-value (µg extract/ml) is the effective concentration which proves 50% of activity, was calculated for each assay. Statistical comparisons were done with Student's test. Differences were considered to be higly significant at $P < 0.01$ and significant at $P< 0.05$.

Results and discussion

Determination of antioxidant activity

Scavenging effect on DPPH radical.

The antioxidants react with DPPH°, a stable purple colored free radical and convert it into colorless α-α-diphenyl-β-picryl hydrazine. The extent discoloration indicates the amount of DPPH scavenged (Ohinishi *et al.*,1994).As shown in Figure 1, the DPPH radical scavenging activities of various investigated extracts from leaves of *P. jaubertii* were in order of AcOEt extract (96.87%) > *n*-BuOH extract (63.62%) > $CHCl_3$ extract (33.78%) at concentration 50 µg/ml. These data suggested that both AcOEt and BuOH extracts of *P. jaubertii* have a remarkable ability to scavenge radicals with IC_{50}-value (7.17 ± 0.82 µg/ml and 20.06 ± 1.86 µg/ml respectively) (Table 1). The AcOEt extract (96.45%) exhibited potent radical scavenging activity even at lower concentration 20 µg/ml which was the dose dependent manner.

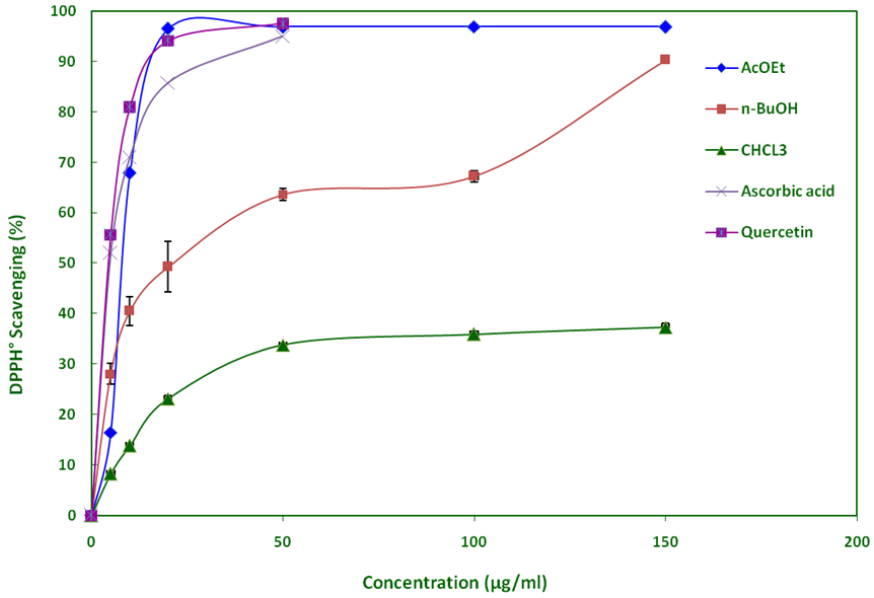

Fig. 1: DPPH radical-scavenging activities of different extracts of the leaves of *P. Jaubertii* and standards. Each value represents a mean ± SD (n=3), P<0.05.

Reducing power assay

The reducing power assay measures the electron-donating capacity of antioxidant using potassium ferricyanide reduction method (Hollman, 2001). The reducing power results of various extracts of leaves of *P. jaubertii* and standard references which are presented in Figure 2, followed the order : ascorbic acid (1.7) > quercetin (1.3) > AcOEt extract (1.24) > *n*-BuOH extract (0.9) > $CHCl_3$ extract (0.15) at 400 µg/ml. Regarding to IC_{50} -values (233.45 ± 1.66 µg/ml and 275.07 ± 17.43 µg/ml) both AcOEt extract and *n*-BuOH extract reduced potentially the ferric ion/ferricyanide to the ferrous form (Fe^{2+}). Previous study reported that reducing power of medicinal plants prevents liver injury by inhibiting formation of lipid peroxides (Seeram and Nair.,2002).

Inhibition of lipid peroxide formation induced by Fe^{2+}/ascorbic acid system

Free radicals (OH°· and $O°^{-}{}_2$) mediated peroxydation unsaturated fatty acid within in the membrane lipid bilayer (Heijnen *et al*.,2002). which can be induced in liver homogenate by Fe^{2+}/ascorbate system. The results shown in Figure 3, revealed that both AcOEt (89.34 %) and *n*-BuOH (65.64 %) extracts proved a good protection and reduce MDA generated by Fe^{3+}/ascorbate at concentration 200 µg/ml. The AcOEt extract (89.34 %) was found to be a better inhibitor of lipid peroxidation compared to the reference standard ascorbic acid (72.34%). Investigated $CHCl_3$ extract showed a moderate protection (45.69 %) activity but even at the highest concentration (600 µg/ml). The propagation of lipid peroxydation is broken by either enzymatic inactivation of reactive oxygen species involved in chain reaction or non-enzymatic reactions (Heijnen *et al*.,2002).due to the intervention of free radical scavenger and antioxidant compounds probably present in the investigated extracts.

Hydroxyl *radical scavenging activity*

OH radical is the most reactive free radical in biological system and it can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions, such as copper and iron (Valko *et al*.,2006). The OH radical scavenging activity was carried out by measuring the competition between deoxyribose and the various extracts from leaves of *P. jaubertii* for hydroxyl radicals generated from Fe^{3+}/ascorbate/EDTA/H_2O_2 system. Figure 4 showed that both extracts AcOEt (63.3 - 84.13 %) and *n*-BuOH (50.94 - 72 %) at the concentration ranged from 200 to 400 µg/ml, were considerably more active than $CHCl_3$ extract (11.65 -28.66 %). The hydroxyl radical scavenging capacity of the investigated extracts compared to standard references was in order to quercetin (83.51%) > AcOEt (63.32 %) > *n*-BuOH (50.94 %) > $CHCl_3$ (11.65 %) at concentration 200 µg/ml. The ability of both AcOEt and *n*-BuOH extracts of leaves of *P. jaubertii* to quench hydroxyl radicals which are implicated in some diseases, revealed the presence of antioxidant compounds in this plant.

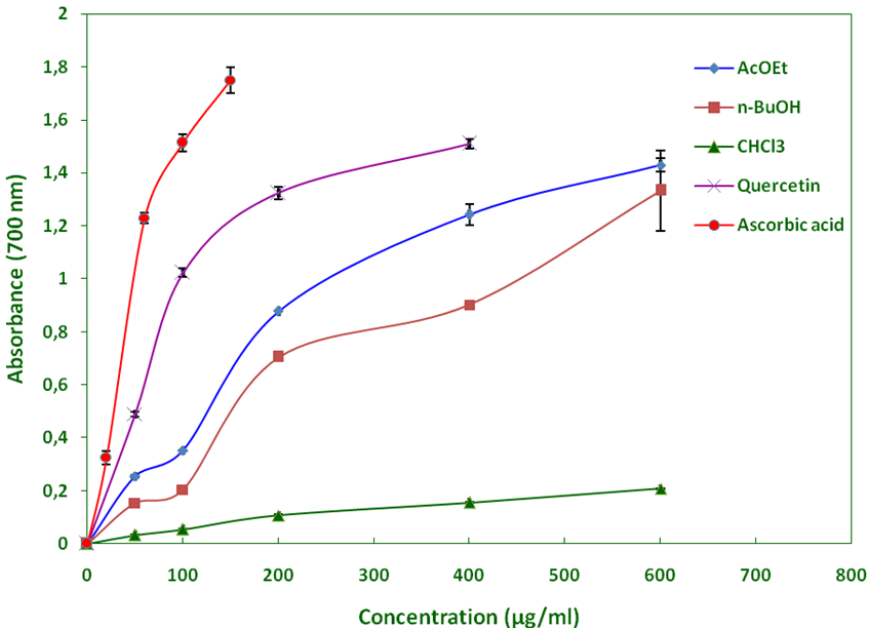

Fig. 2: Reducing power of of different extracts of the leaves of *P. Jaubertii* and standards. Each value represents a mean ± SD (n=3), P<0.05.

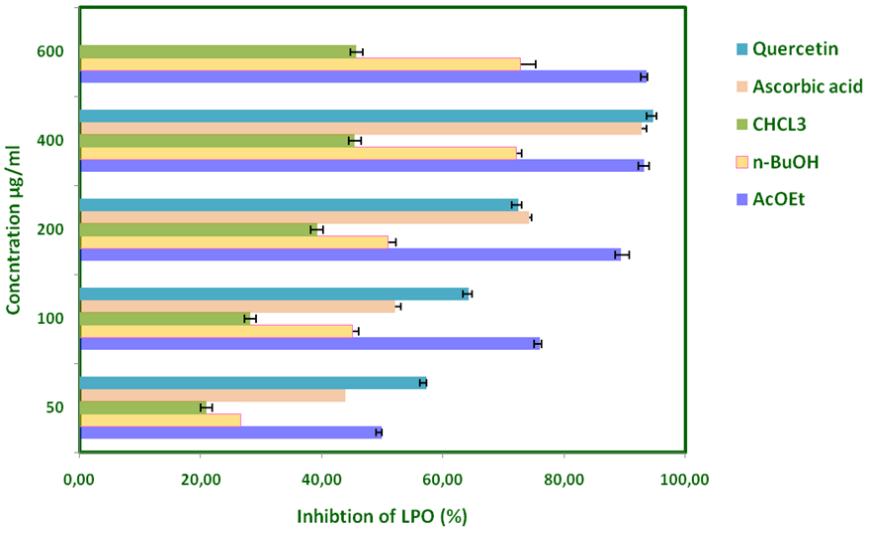

Fig. 3: Inhibition of lipid peroxidation activities of different extracts of the leaves of *P. Jaubertii* and standards. Each value represents a mean ± SD (n=3), P<0.05.

Ferrous-chelating ability

The iron-chelating capacity test measures the ability of antioxidants to compete with ferrozine in chelating ferrous ion (Afanas'ev *et al*.,1989). This assay as shown in Figure 5, indicated that both AcOEt and *n*-BuOH extracts of *P. jaubertii* interfered with the formation of activity and captured ferrous ions before ferrozine. As it can be deduced from Figure 5, these two extracts, AcOEt (81.46 %) and *n*-BuOH extract (63.65 %) had the highest ferrous ion chelating activity at a concentration 400 µg/ml. Although the $CHCl_3$ extract did not show ion chelator activity even at the highest concentration, the antioxidant compounds contained in AcOEt and *n*-BuOH extracts of *P. jaubertii* probably inhibited interaction between metal and lipid through formation of insoluble complexes with ferrous ion (Heijnen *et al*.,2002).

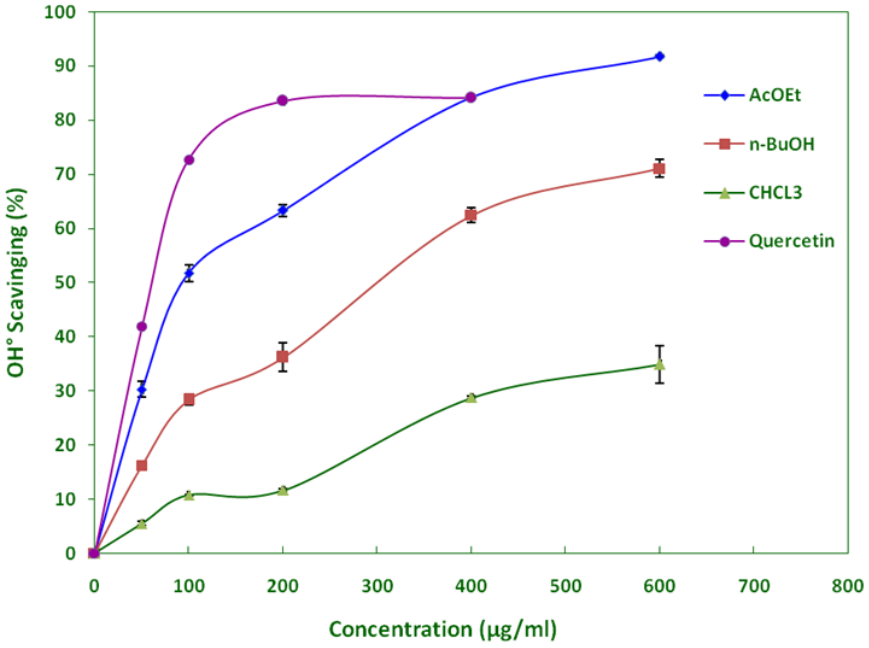

Fig. 4: OH radical scavenging activities of different extracts of the leaves of *P. Jaubertii* and standards. Each value represents a mean ± SD (n=3), P<0.05.

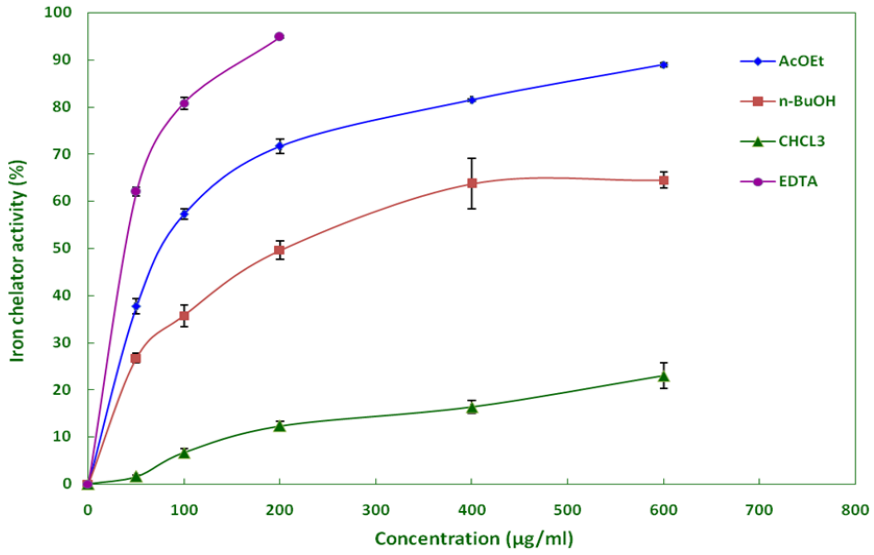

Fig. 5: Ferrousion chelating ability of different extracts of the leaves of *P. Jaubertii* and standards. Each value represents a mean ± SD (n=3), P<0.05.

Polyphenol contents and IC_{50} -values of antioxidant activities

Table 1: Polyphenol contents and IC_{50} -values of antioxidant activities of extracts from leaves of *P. jaubertii*.

Extract and standard reference	IC_{50} (µg/ml)					Total Polyphenols (mgGAE/g)	Total flavonoids (mgQE/g)
	DPPH°	Iron-chelating	OH°	LPO	Reducing Power		
AcOEt	7,17±0,82**	79,45±3,59**	105,63±2,36**	31,19±3,74**	233.45±1.66**	322,98±33,76**	1 159,80±22,11**
n-BuOH	20,06±1,86*	216,58±31,70*	259,84±14,90*	148,87±9,58*	275.07±17.43**	77,83±6,79*	19,52±3,68**
$CHCl_3$	457,55±31,86	22794,077±9911.69	2889,08±877,33	xxx	256±2.73	1,08±0,07	0,19±0,01
Quercetin	2,47±0,05**	-	50,0±80,70**	39,67±1,71**	124.49±0.37**	-	-
Ascorbic acid	3,74±0,02**	-	-	74,10±2,16**	50.05±11.33**	-	-
EDTA	-	29.16±1.71**	-	-	-	-	-

Comparison between extracts, ,** highly significant at $P < 0.01$, * significatif at $P<0.05$
(GAE)/g extract : mg gallic acid equivalents / g extract, (QE)/g extract : mg quercetin equivalents / g extract,
AcOEt compare to *n*-BuOH and $CHCl_3$, * significant at $P < 0.05$, *n*-BuOH compare to $CHCl_3$

According to the IC_{50}-values presented in Table 1, the AcOEt extract from leaves of *P. jaubertii* have an important role in scavenging abilities of various radicals and IC_{50}-values of antioxidant activities followed the order : DPPH° (7.17 µg/ml) > LPO (31.19 µg/ml) > iron-chelating (79.4 µg/ml) > OH° (105.63 µg/ml) > reducing power (233.45 µg/ml) compared to the IC_{50} values of antioxidant activities of *n*-BuOH extract : DPPH° (20.06 µg/ml) > LPO (148.87 µg/ml) > iron-chelating (216.58 µg/ml) > OH° (259.84 µg/ml) > reducing power (275.07 µg/ml). The AcOEt extract which had the highest content of polyphenols and flavonoids was the most powerful compared to *n*-BuOH extract. Previous investigations reported that the beneficial effect of plants is mainly associated with the antioxidant activity of their phenolic compounds (Pietta., 2000). It was also revealed that the extracts obtained by polar organic solvents (acetone and methanol) were potentially active than those obtained by umpolar organic solvents (chloroform and petroleum ether) (Çakir *et al*., 2006). This supports the idea that the polar compounds (flavonoids) present in the extract are mainly responsible for its antioxidant activity (Bravo, 1998).

Conclusion

The results obtained in this study clearly showed that both AcOEt and *n*-BuOH extracts from the leaves of *Pulicaria jaubertii* possess antioxidant activity. The AcOEt extract exhibited a strong antioxidant activity and had the most potent scavenging abilities of various radicals which may be caused by the high content of polyphenols and flavonoids. The very strong antioxidant activity of the *P. jaubertii* suggests that the extracts obtained by polar solvents from the leaves could be used as an effective natural source of antioxidant and food additives.

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