September 2010 ISSN : 0974 - 7508 Volume 6 Issue 3

Trade Science Inc.

Natural Products

An Indian Journal

Review

NPAIJ, 6(3), 2010 [118-124]

Total phenolic contents and antioxidant activity of organic fractions from *Capparis spinosa* and *Limoniastrum feei*

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Received: 8th May, 2010 ; Accepted: 18th May, 2010

ABSTRACT

In recent years, interest in finding new sources of natural antioxidants has increased, especially that of medicinal plants which are mainly required in the pharmaceutical field. The study of antioxidant activity has become an inescapable in the search for new bioactive substances. Phenolic compounds appear to be targeted by researchers. Our work aim is to evaluate the antioxidant capacity of phenolic compounds extracted from two Algerian medicinal plants: *Capparis spinosa* and *Limoniastrum feei*. The two plants of this study presented average content of phenolics and flavonoids compounds. The antioxidant activity of the various organic fractions was evaluated using different antioxidant assays, including: reducing power and DPPH free radical scavenging activity. The results showed that the ethyl acetate fraction (fraction rich in mono and di-glycosids) had a high antioxidant activity than the butanolic fraction (fraction rich in polyglycosids). In addition, the organic fractions of different parts of *Limoniastrum feei* presented an high potent to scavenging DPPH radical and reducing power.

KEYWORDS

Medicinal plants;
Capparis spinosa;
Limoniastrum feei;
Phenolic compounds;
Antioxidant activity;
Reducing power.

INTRODUCTION

The last decades were marked by the special interest in the development of medicinal plants as a source of natural bioactive substances. As a result, many studies are, increasingly, the therapeutic effects of antioxidants of natural origin. Because of their abundance in nature and their uses by indigenous people for healing, some plants were placed in the medical world.

However, this seems inexhaustible source, since only a small portion of the 400,000 known plant species has been investigated both phytochemical and pharmacological, and each species may contain up to several thousands of different constituents[19].

Natural substances from plants have multiple interests used in industry (food, cosmetics and dermopharmacy industries). Among these compounds secondary metabolites which are mainly reflected in the therapeutic area. Recent works aimed at isolating new substances from plants and find other applications in various fields. However, modern medicine still uses a large proportion of drugs of plant origin and research found in plants of new active drugs or raw materials for chemical synthesis. Natural and synthetic antioxidants

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have been shown to enhance product stability, quality and shelf. Many research works have mentioned the disadvantage of synthetic antioxidants;[11,12,24,27]. Indeed, the use of synthetic antioxidant in food products has decreased due to their instability, as well as their possible toxic and carcinogenic effects on health[11]. Therefore, research into the determination of natural antioxidant sources is important. In the search for sources of natural antioxidants, some medicinal plants have been extensively studied for their antioxidant activity and radical scavenging activity in the last years[7]. Nowadays, research has focused on medicinal plants to extract new natural antioxidants that can replace synthetic additives[9]. Phenolics are an important class of secondary plant metabolites possessing an umpressive array of pharmacological activity. One of the more prominent properties of the phenolics is their excellent radical scavenging ability[10]. This one is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers agents, and they have also metal chelating potential[23]. *Capparis spinosa* L. (Capparidaceae) is a plant from the dry regions in west or central Asia and widely grown particularly in the Mediterranean basin. From ancient times, the floral buttons of *C.spinosa* (capers) were employed as flavouring in cooking and are also used in traditional medicine for their many therapeutic effects (TABLE 1). Previous chemical studies on *C. spinosa* have shown the presence of alkaloids, lipids, polyphenols, flavonoids, indole and aliphatic glucosinolates[21]. *Limoniastrum feei* (Plumbaginaceae), is one of the medicinal plants used for various medicinal uses in Algerian folk medicine (TABLE 1). The plant is native to southeast of Algeria, northern Africa[2].

The aim of present works is to study *in vitro* antioxidant activities of the organic frations of *L. feei* and *C.spinosa* using ferric reducing antioxidant power (FRAP) and DPPH radical scavenging assays. In addition, the total content of phenolics and flavonoids from plant extracts were also measured.

TABLE 1 : Popular use of the selected plants in folk medicine.

Plant name	Popular use in folk medicine
Capparis spinosa (Capparidaceae)	Analgesic, laxative, astringent, diuretic, emmenagogue and vermifuge (Shahina, 1994). Also used in the treatment of rheumatism, scurvy, splenomegaly and toothache. The stems are used for dysentery (Duke *et al.,* 2003). Capers have been suggested for atherosclerosis and sciatica, especially in North Africa. The buds and roots are used as disinfectants kidney, tonic for arteriosclerosis and as compresses for the eyes (Batanouny *et al.,* 1999). Used also for traitemetent of viral hepatitis
Limoniastrum feei (Plumbaginaceae)	Used to treat gastric infections, bronchitis and stomach infection[3]. In some regions, this plant is used in the treatment of persons who have been biting of scorpions, and has several uses by indigenous people who deserve to be studied and published

MATERIAL AND METHODS

Chemicals

1,1-diphenyl-2-picryl-hydrazyl (DPPH), potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), Folin-Ciocalteu's phenol reagent, Butylated hydroxyanisol (BHA), Butylated hydroxytoluene (BHT) and ascorbic acid were purchased from Fluka (Switzerland). Sodium carbonate and sodium hydroxide were from Merk (Germany). $FeCl_3$ was from Sigma Chemical Co (Germany). Methanol was from Biochem (Chemopharma, UK). All other chemicals and solvents used were of analytical grade available commercially.

Plant materials

Species selected: *C.spinosa* and *L.feei* were collected in their natural habitat in the region of Ain Ouarka ((Region of Naâma, south-west of Algeria) during the months of November and December 2007, and dried away from direct sunlight. The plants were identified at the laboratory of Ecology and Management of Natural Ecosystems of the University of Tlemcen (Algeria). Dried plant materials were then crushed into a mortar and stored at very low temperature until further use.

Sample preparation

A powder (2g) of each part of the plants (leaves and stems) was extracted by the mixture methanol-water (70:30, v/v). The preparation is carried under reflux for

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3h (Bekkara et al., 1998). After cooling to room temperature, the methanolic extract is filtered and evaporated under reduced pressure at 60°C using a rotary evaporator (Büchi Rotavapor R-200). The dry residue obtained after evaporation of the methanolic filtrate of each of the two plants studied, were divided between 20 ml of ethyl acetate and the same volume of distilled water in a separating funnel. After decantation of the two phases, the ethyl acetate phase is recovered and the aqueous phase is again divided with 20 ml of n-butanol. The operation is repeated twice for each step. The phases obtained are dried using a rotary evaporator. The dry residues were taken up by a few milliliters of methanol and kept at +4°C. Finally two fractions are obtained: fraction with ethyl acetate (EtOAc) and fraction with n-butanol (BuOH) (Figure 1).

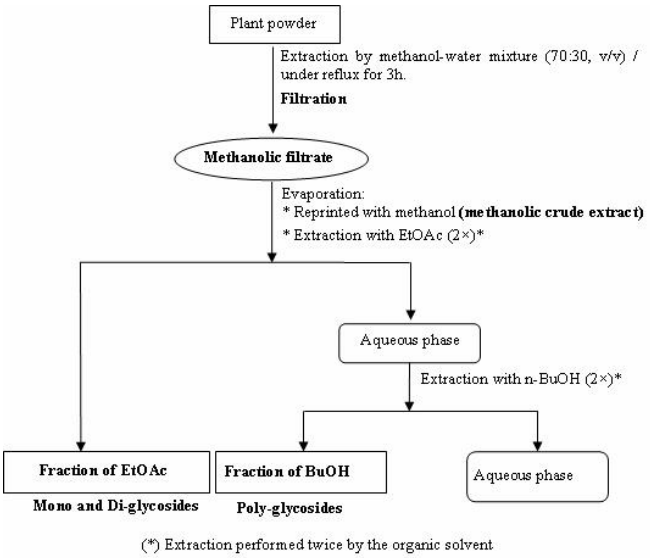

Figure 1 : Monitoring protocol for the extraction of polyphenols and obtaining fractions of ethyl acetate and n-butanol

Total phenolics content

Total phenolics content were estimated by the Folin-Ciocalteu method (Vermerris & Nicholson, 2006). 0.1 ml of the methanolic crude extract was mixed with 2 ml of sodium carbonate (2 %) freshly prepared, the whole was vigorously mixed on a vortex. After 5 min, 100 µl of Folin-Ciocalteu reagent (1N) were added to the mixture, all was left for 30 min at room temperature and the reading is performed against a blank at 750 nm. A calibration curve was performed in parallel under the same operating conditions using gallic acid as a positive control. The results are expressed as mg gallic acid equivalent per gramme of dry extract (mg GAE/g).

Total flavonoids content

The total flavonoid content was determined by a colorimetric method as described in the literature[1]. Each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a $NaNO_2$ solution (15 %). After 6 min, 0.15 ml of aluminum chloride ($AlCl_3$) solution (10 %) was added and allowed to stand for 6 min, then 2 ml of NaOH solution (4 %) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm versus prepared water blank. A calibration curve was performed in parallel under the same operating conditions using catechin as a positive control. Results were expressed as mg catechin equivalent per gramme of dry extract (mg CEQ/g).

Antioxidant activity

Ferric reducing antioxidant power assay

The reducing power of the different part of *L.feei* and *C.spinosa* was determined according to the method of Yang et al.[26]. The EtOAc and BuOH fraction and ascorbic acid were used at differing concentrations (0.1, 0.25 and 0.75mg/ml). One milliliter of each sample was mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 30 mmol/l). The mixture was incubated at 50 °C for 20 min. A 2.5 ml TCA (0.6 mol/l) was added to the mixture, which was then centrifuged for 10 min at 3000 g. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 6 mmol/l), and the absorbance was measured at 700 nm in a spectrophotometer (Jenway 6400).

Determination of the scavenging effect on DPPH radicals

A methanolic solution (50 µl) of each fraction at different concentrations was added to 1.95 ml of DPPH solution (6×10^5 M in methanol) (Atoui et al., 2005). The studied compounds were tested with methanol as control and ascorbic acid as antioxidant references and absorbance at 515 nm was determined after 30 min. The absorbance (*A*) of the control and samples was measured, and the DPPH scavenging activity (SA) in

percentage was determined as follow:

$$SA\ \% = [A_{control} - A_{sample}) / A_{control}] \times 100$$

IC_{50} was obtained graphically from linear regression analysis.

RESULTS AND DISCUSSION

Total penolics and flavonoid content

Determination of total phenolic and flavonoid contents of the methanolic crude extracts of the two plants of this study were done by using Folin–Ciocalteu colorimetric and $AlCl_3$ methods, separately. Total polyphenol contents were estimated with Folin–Ciocalteu colorimetric method. This reagent is reduced during the oxidation of phenols in a mixture of blue oxides of tungsten and molybdenum, the color produced, whose absorption maximum is between 700 and 750 nm, is proportional to the amount of polyphenols present in plant extracts. The results are reported in TABLE 2. The methanolic crude extract of *L.feei* shown a high phenolic content in the two extracts of plant (leave and stem) compared to methanolic extracts of *C.spinosa*, and we can observe that the methanolic crude extract of leaves contained a high phenolic compounds than the crude extract of the stems. In $AlCl_3$ colorimetric method, aluminum chloride forms acid stable complex with the keto and/or the hydroxyl groups in the A or C ring of flavonoids[1]. The results, as presented in TABLE 2, show that the methanolic crude extract of leaves of *L.feei* contained high flavonoids compounds compared to the crude extract of the stems and methanolic extract of *C.spinosa* for the two parts. The lowest content was that of the methanolic extract of stems of *C.spinosa* (Figure 2). Concerning *L.feei*, there are very few publications that are made regarding the levels of polyphenols and flavonoids. Indeed, recently, the publication by Chaabi et al.[5] reports that *L.feei* contains seven polyphenolic constituents: gallic acid, myrciaphenone A, myricetin-3-*O*-*β*-galactopyranoside, epigallocatechin gallate, myricetin 3-*O*-*α*-rhamnopyranoside, quercetin and myricetin. The phytochemistry investigation of the water-acetone extract of twig part of *L.feei* led to isolation of four flavonoids. The structures of these compounds were identified as: 6,3',4'-tri-methoxy 3,5,5'-trihydroxy flavonol, 3-(6''-malonyl 2''-ramnosyl glucosil) 6,3',4'-trimethoxy 5,5'-dihydroxy flavonol, tetraacetate 7-dihydroxy-4'-methoxy 8-O-β-glucopyranoside isoflavone and tetraacetate 7,4'dimethoxy 8-O-β-glucopyranoside isoflavone[3]. This plant contains large amounts of phenolics (phenolic acids, tannins, flavonoids, flavonols, isoflavone) reflected by the high levels of this chemicals found in this study concerning phenolics and flavonoids.

A study by Proestos et al.[22] on *C.spinosa* from Greece, reports that this species contains phenolic acids (caffeic, vanillic, ferulic and *p*-Coumaric acids) and flavonoids (quercetin and rutin), but at low concentrations. Another study shows that the genus *Capparis* is an important source in flavonols and flavonoids in particular, with levels of about 5.18 mg per gram of fresh plant material[16]. Giuffrida et al.[14] contradicts the results published by the study of Inocencio et al.[16] and revealed low levels of flavonoids in the plant studied. *C.spinosa* presented average contents concerning phenolic compounds and flavonoids, this is consistent with work published on this plant[14,16,22].

TABLE 2 : Total phenolic[a] and total flavonoid[b] of methanolic crude extracts of *L.feei* and *C.spinosa*

		Total phenolics (mg GAE/g)	Total flavonoids (mg CEQ/g)
Limoniastrum feei	Leaves	98.24 ± 4.56	60.35 ± 5.56
	Stems	83.97 ± 3.25	36.35 ± 4.92
Capparis spinosa	Leaves	51.04 ± 2.22	43.22 ± 2.42
	Stems	12.01 ± 1.06	07.06 ± 0.09

Each value represents the mean ± SD (n = 2).

[a] Total phenolic content was expressed as mg gallic acid equivalents/g dried extract.

[b] Total flavonoid content was expressed as mg catechin equivalents/g dried extract.

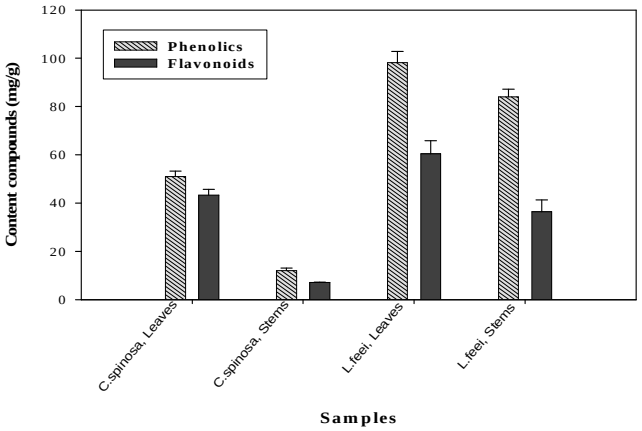

Figure 2 : Total phenolics and flavonoids content of methanolic extracts of *L.feei* and *C.spinosa*

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Antioxidant activity

Ferric reducing antioxidant power assay

Reducing power is one mechanism for action of antioxidants[17]. The presence of reduction in a given environment causes the reduction of Fe^{3+} ferricyanide complex to form Fe^{2+}. Indeed, the formation of Fe^{2+} can be followed spectrophotometrically by measuring the density of the blue complex of ferrous reaction mixture at 700 nm. An increase in absorbance indicates increased reducing power of extracts tested[20]. Figure 3 shows the reductive capability of EtOAc and BuOH fractions of *L.feei* and *C.spinosa* compared to the reducing power of ascorbic acid as standard.

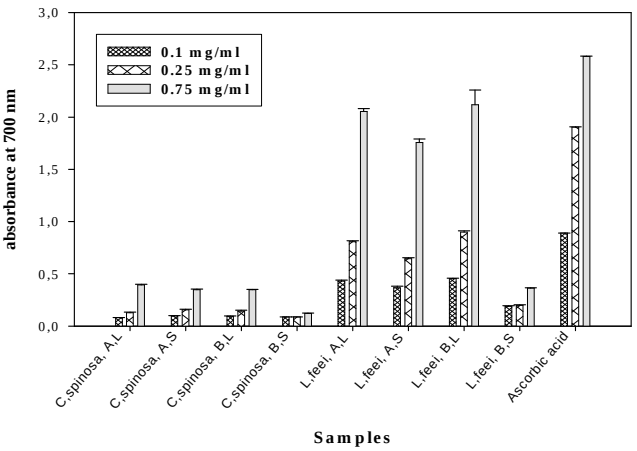
We note that the EtOAc fraction of leaves of *C.spinosa* introduced more reducing power relative to other fractions, and both fractions; EtOAc fraction of stems and BuOH fraction of the leaves, had the same effect against the reduction of iron and that the BuOH fraction of the stems presented a very low activity observed values of optical densities not exceeding 0.3.

Concerning *L.feei*, we note that at all fractions for both sides studied presented significant activities towards the reduction power. Indeed, the leaves part presented a power almost comparable to that of ascorbic acid with high optical densities significantly, while the BuOH fraction of the stems was the exception of very low optical densities. We can see clearly that the ascorbic acid has a remarkable activity to reduce iron, reflected by the high absorbance (A) obtained at different concentrations of it.

We note that at concentration of 0.75 mg/ml, the fraction BuOH and EtOAc fraction of the leaves *L.feei* have the highest activity to reduce iron, more or less similar to that of ascorbic acid at the same concentration (A = 2.07 for EtOAc fraction, A = 2.11 for BuOH fraction, and A = 2.58 for ascorbic acid). The EtOAc fraction of the stems of the plant has a reducing power activity similar to that of ascorbic acid at the concentration of 0.25 mg/ml.

We can classify the power reduction of the different fractions of the two plants studied as follows: Ascorbic acid> BuOH fraction of leaves *L.feei*> EtOAc fraction of leaves *L.feei*> EtOAc fraction of stems *L.feei*> EtOAc fraction of leaves *C.spinosa*> BuOH fraction of stems *L.feei*> EtOAc fraction of stems *C.spinosa*> BuOH fraction of leaves *C.spinosa*> BuOH fraction of the stems *C.spinosa*.

Reducing power ability of a compound may serve as a significant indicator of its potential antioxidant activity[26]. Many publications have reported that there is a direct correlation between antioxidant activities and the power reduction components of several plants[28]. The results obtained for a plant extract which has a high activity, suggesting that this one has a remarkable power to give electrons to reactive free radicals (or reactive species), converting them into non-reactive and more-stable, ending the chain reaction of free radicals. Browsing results, the two studied fractions of EtOAc and BuOH are almost the same activities to reduce iron. We noticed that *C.spinosa* presented moderate antioxidant activity compared to ascorbic acid. However, *L.feei* presented an activity comparable to that of ascorbic acid, this can probably be explained by the presence of compounds capable to reduce iron.



AL: EtOAc fraction of Leaves **AS: EtOAc fraction of stems**
BL: BuOH fraction of Leaves **BS: BuOH fraction of stems**

Figure 3 : Reducing power activity of the EtOAc and BuOH fractions of *L.feei* and *C.spinosa*

DPPH radical scavenging

The radical $DPPH^{\cdot}$ is one of the substrates most commonly used for rapid assessment and direct antioxidant activity because of its stability in radical form and simplicity of the analysis[4]. The model for scavenging stable DPPH free radicals can be used to evaluate the antioxidative activities in a relatively short time. The absorbance decreases as a result of a colour change from purple to yellow as the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H molecule[8]. The effect of antioxidants

on DPPH radical-scavenging was thought to be due to their hydrogen-donating ability. The preparations were able to reduce the stable free radical, DPPH, to the yellow-coloured 1,1-diphenyl-2-picrylhydrazyl.

The fractions studied of *L.feei* and *C.spinosa* were tested for their antioxidant scavenging effects on DPPH radical and their activity was compared to ascorbic acid used as antioxidant reference. IC_{50} or inhibitory concentration 50% (also called Efficient EC_{50}), is the concentration of sample required to reduce 50% of DPPH· radical. The IC_{50} is calculated graphically by linear regression graph plots. The results obtained are given in TABLE 3. The IC_{50} value of ascorbic acid found (2.97 µg/ml), is close to the IC_{50} values found by Conforti et al.[7] (2 µg/ml) and Chew et al.[6] (3.82 µg/ml). By comparing the IC_{50} of the different plants studied in relation to ascorbic acid, we noticed a high antioxidant activity of the EtOAc fraction of the two parts (leaves and stems) of *L.feei*. The same goes for the BuOH fraction of the leaves of the same plant.

In our study, the EtOAc fraction of the leaves of *L.feei*, presented an antioxidant activity comparable to that of ascorbic acid, this suggests that this plant has several compounds possess high antioxidant activity. For *C.spinosa*, we recorded an average antioxidant activity against DPPH· scavenging. The results found for the plant *C.spinosa* are consistent with the results found by Hamed et al.[15], who worked on other species of the genus (*C.cartilaginea* and *C.deserti*). By comparing the antioxidant activity of the two fractions studied, we observed that the EtOAc has more activity compared to the BuOH fraction. Our results confirm those published by Tian et al.[25] and Fabri et al.[13]. Even if the comparison between different methods for evaluating the antioxidant activity *in vitro* is difficult[18], we noticed that there is some conformity between the results obtained by FRAP analysis and found by the DPPH· method. Tian et al.[25] have found a correlation between the results obtained by these two tests.

TABLE 3 : The IC_{50} value[a] of EtOAc and BuOH fractions of *L.feei, C.spinosa* and ascorbic acid

Plantes et parties étudiées		EtOAc fraction	BuOH fraction
		IC_{50} values	
Capparis spinosa	Leaves	13.95	34.59
	Stems	55.06	498.4
Limoniastrum feei	Leaves	3.08	5.25
	Stems	5.20	69.64
Ascorbic Acid		2.97	

Each value represents the mean of duplicate assays.

[a] IC_{50} values were expressed as µg/ml (final concentrations).

CONCLUSION

Our work aim was to study the antioxidant fractions of EtOAc and BuOH from different parts of two medicinal plants by the capacity to scavenging the radical DPPH· and their reducing power activity to find the fraction that represents the most activity. This approach has led us to infer from the results that the fractions EtOAc has the advantage of the more active compared to BuOH fractions, these results are fully verified by the literature. The fraction of EtOAc from the leaves of *L.feei* presented a relatively high antioxidant activity, with a value of IC_{50} of about 3.085 µg/ml, comparable to that of ascorbic acid (IC_{50} = 2.97 µg/ml), while *C.spinosa* has presented a moderate antioxidant activity, in the case of the radical DPPH· scavenging. For FRAP analysis, we found an interesting activity of the species *L.feei* comparable to that of ascorbic acid. Therefore, the phenolic compounds of *L.feei* presented an interest antioxidant activity.

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