

## Research Letter

# Comparison between polyphenol contents and antioxidant activities of different parts of *Capparis spinosa* L

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**ABSTRACT:** **Introduction:** *Capparis spinosa* L., Capparidaceae is a shrub growing widely in Algeria. Aqueous and methanolic extracts of five parts of the plant were studied for their polyphenol and flavonoid contents. These extracts were also evaluated for their antioxidant and anti-lipid peroxidation effects. **Material and Methods:** determination of polyphenols and flavonoids was carried out using colorimetric methods. Antioxidant and anti-lipid peroxidation of extracts were performed using the established DPPH and beta carotene/linoleic acid tests. **Results:** The methanolic extracts contained higher total polyphenols and flavonoids than the aqueous extracts. The leaves and flowers were rich in polyphenols and/or flavonoids whilst the roots were relatively poor in these components. Furthermore, all extracts had anti-lipid peroxidation and antioxidant effects with higher levels in the flowers and the leaves ( $I\% = 82.78 \pm 2.64\%$  and  $80.94 \pm 1.57\%$ , respectively). Seeds have an important effect ( $I\% = 64.02\%$ ) followed by fruits then roots ( $I\% = 40.62\%$  and  $21.24\%$ , respectively). **Conclusion:** These results demonstrated the antioxidant and anti-lipid peroxidation of all parts of *Capparis spinosa* and give a comparative data on the extracts of different parts that can be used in the diseases where inhibition of oxidative stress is required.

**KEYWORDS:** *Capparis spinosa*, antioxidant, methanolic extract, aqueous extract, lipid peroxidation

## INTRODUCTION

The common caper (*Capparis spinosa* L., Capparidaceae) is a species of Mediterranean shrub very important for the natural surroundings and economy of Algeria.<sup>[1]</sup> *Capparis spinosa* is a evergreen shrub which covers soil surfaces and produces a deep root system.<sup>[2]</sup> As such, *Capparis spinosa* appears to be a suitable candidate for combating desertification.<sup>[3]</sup> Being a rich source of vitamins and minerals, caper buds are essential component of Mediterranean cuisines.<sup>[4]</sup> *C. spinosa* is said to be native to the Mediterranean basin, but its range stretches from the Atlantic coasts of the Canary Islands and Morocco to the Black Sea to the Crimea and Armenia, and eastward to the Caspian Sea and into Iran. It

grows in North Africa, Europe, West Asia, Afghanistan, and Australia. In India it grows from Punjab and Rajasthan to the Deccan Peninsula.<sup>[5]</sup> *C. spinosa* possesses a great number of medicinally important compounds and has an ethnobotanical importance.<sup>[6]</sup> The root bark is used as an analgesic in the treatment of gastrointestinal conditions,<sup>[7]</sup> gout, rheumatism, and as a laxative and expectorant for pulmonary diseases.<sup>[8]</sup> Infusions and decoctions from caper root bark have been traditionally used for dropsy, anaemia.<sup>[9,10]</sup> The flower buds are used as a laxative and to stimulate appetite.<sup>[11]</sup> An infusion of stem bark and root is used to treat diarrhea in folk medicine.<sup>[7]</sup> Externally, caper was used to treat eye infections and in the prevention of cataracts.<sup>[12]</sup> The leaves, folded, with a little water or rubbed, are used for external use against insect bites and hives.<sup>[7]</sup> The fruits were used to treat arthritis,<sup>[13]</sup> ear infections, coughs, expelling worms from the stomach, and diabetes, whilst the flower buds and roots are used as disinfectants, diuretic, tonic, for the treatment of arteriosclerosis and as a compress for the eyes.<sup>[14]</sup> Aqueous fruit extracts have a diuretic effect accompanied by an increase in the concentration of  $\text{Na}^+$ ,

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$K^+$  and  $Cl^-$  in the urinary excretion in rats.<sup>[15]</sup> It has been shown that repeated doses of oral aqueous extract resulted in a potent anti-hyperglycemic effect and blocked obesity.<sup>[16]</sup> Other studies<sup>[17,18]</sup> have shown that aqueous extracts of *Capparis spinosa* (20 mg/kg) have potent activity on the reduction of plasma cholesterol, triglycerides and glucose in normal and severe hyperglycemia. An experiment conducted on the effect of a methanolic extract of flower buds on inflammation showed that this extract is able to antagonize the effects of IL-1beta in a more pronounced manner than indomethacin.<sup>[19]</sup> Ethanolic extracts of root bark has shown dose-dependent protective effects against liver cell damage caused by  $CCl_4$ .<sup>[19]</sup> The leaf extracts can stimulate melanogenesis in a dose-dependent manner without cytotoxicity by increasing tyrosinase protein expression.<sup>[20]</sup> In our previous study<sup>[21]</sup> we have examined the antioxidant effect of extracts of the root and aerial parts of *C. spinosa*. The present study aims to investigate the comparative antioxidant effects of either methanolic or aqueous extracts from different parts: root, leaves, fruits (caper), flowers and seeds of *C. spinosa* and their possible relationship with total contents of polyphenols and flavonoids.

## MATERIAL AND METHODS

### Plant material

*Capparis spinosa*, was collected from the region of Beni-Aziz, Wilaya (Department) of Setif northeast of Algeria between May and July 2009, and was authenticated by Pr H. Laouar (University Ferhat Abbas Setif). A voucher specimen was deposited at the Laboratory of Botany, Department of Vegetal Biology and Ecology, Faculty of Nature and Life Sciences. The extraction of flavonoids was carried out according to a previously described method<sup>[22]</sup> with slight modifications. Dried plant material was ground in a Warring blender and subsequently mixed with a 10–20 volumes of 85% aqueous methanol. The slurry was placed at room temperature for one week and the extract was filtered through a Buchner funnel. The methanol was removed by rotary evaporation.

To obtain the aqueous extracts, 10 g of the powder of each of the different plant parts (roots, leaves, flowers, seeds and fruits) was mixed with 100 mL of distilled water, heated for 15 min, and stirred overnight at 4°C. The aqueous extract was filtered through glass wool to remove particles. The filtrate was then lyophilized and stored at –20°C until use.

### Determination of total polyphenols and flavonoids

Total polyphenols were measured using Prussian blue assay method described by Price & Butler.<sup>[23]</sup> and modified

by Graham.<sup>[24]</sup> Phenolic contents were expressed as gallic acid equivalents. Briefly, 0.1 mL of each *Capparis spinosa* extract was dissolved in methanol and 3 mL distilled water were added and mixed. One mL of  $K_3Fe(CN)_6$  (0.016 M) was added to each sample followed by the addition of 1 mL of  $FeCl_3$  (0.02 M dissolved in 0.1 M HCl) and immediately mixed using a vortex. After the addition of the reagents to the sample, 5 mL/mL of stabilizer (30 mL of 1% gum arabic, 30 mL of 85%  $H_3PO_4$  and 90 mL distilled water) was added to the sample and mixed. The absorbance was measured at 700 nm using a UV/VIS-8500 Techom spectrophotometer. The amount of total polyphenols in the various extracts was determined from a standard curve of gallic acid ranging from 0.00 to 200  $\mu$ g/mL.

Flavonoids were measured by the  $AlCl_3$  method described previously,<sup>[25]</sup> and expressed as quercetin equivalents. One mL of the plant extracts samples was dissolved in methanol, 1 mL of  $AlCl_3$  (2%) in methanol was added. After incubation for 10 min, the absorbance was measured at 430 nm.

### Antioxidant activity determination with DPPH assay

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-coloured methanol solution of 2,2'-diphenylpicrylhydrazyl (DPPH). This spectrophotometric assay used the stable radical DPPH as a reagent.<sup>[26,27]</sup> Fifty  $\mu$ L of various concentrations of the extracts in methanol were added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition percent (I%) of free radical by DPPH was calculated according to the following equation:

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

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 tested compound. Extract concentration providing 50% inhibition ( $IC_{50}$ ) was calculated from the plot of inhibition percentage against the extract.

### $\beta$ -carotene-linoleic acid assay

The antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation.<sup>[28]</sup> A stock solution of  $\beta$ -carotene-linoleic acid mixture was prepared as follows: 0.5 mg  $\beta$ -carotene was dissolved in 1 mL of chloroform (HPLC grade) and

25  $\mu$ L linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 mL distilled water saturated with oxygen (30 min 100 ml/min) were added with vigorous shaking. 2.5 mL of this reaction mixture were dispensed into test tubes and 350  $\mu$ L of the extracts (2 g/L) were added and the emulsion system was incubated for 48 h at room temperature. The same procedure was repeated with the synthetic antioxidant BHT, as positive control. After the incubation period, absorbances of the mixtures were measured at 490 nm.

### Statistical analysis

All samples were analyzed in triplicate and data were expressed as means  $\pm$  SD using InStat software (Graphpad, San Diego, California). Analysis of variance (ANOVA) was used to test differences between groups. The Tukey-Kramer multiple range test was used to determine the significance of differences between the mean values of the treatment groups at the level of  $p \leq 0.05$ .<sup>[29]</sup>

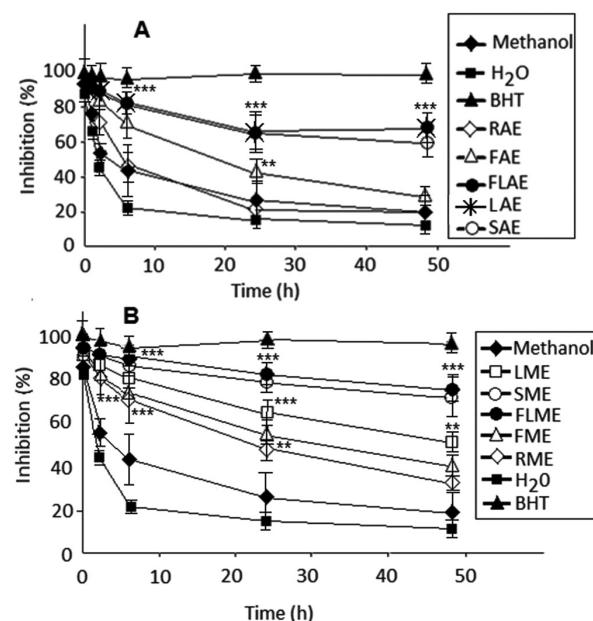
## RESULTS AND DISCUSSION

### Total phenolic and flavonoid contents

The amounts of phenolic and flavonoid compounds in extracts of different parts of *C. spinosa* are presented in Table 1. Total phenolic contents were expressed as mg gallic acid equivalents per gram of dry weight (mg GA-Eq/g) and total flavonoids contents as mg quercetin/rutin equivalents per gram dry weight (mg Q-Eq/g). In the aqueous extract, leaves contain the highest amount of polyphenols ( $56.98 \pm 14.24$  mg GA-Eq/g), followed by seeds ( $35.85 \pm 2.32$  mg GA-Eq/g), flowers ( $34.14 \pm 4.61$  mg GA-Eq/g), roots ( $15.46 \pm 6.86$  mg GA-Eq/g) then fruits ( $7.15 \pm 0.63$  mg GA-Eq/g). Phenolic compounds are commonly found in plants. They have been reported to have many biological effects, including antioxidant activity.<sup>[30]</sup>

### Antioxidant assay using $\beta$ -carotene-linoleate model system

Used at a same concentration of 2 mg/mL, the extracts showed marked antioxidant activity compared to BHT (butylated hydroxytoluene), a synthetic antioxidant which inhibited 98.88% of linoleic acid peroxidation. The root extract showed very weak inhibition compared to other extracts. The variation in absorbance with the time of incubation of different extracts are presented on the figure 1 A, B.



**Figure 1.** Antioxidant capacities expressed as % of inhibition of linoleic acid peroxidation in the presence of BHT and different extracts *Capparis spinosa*. A: aqueous extracts, B: methanolic extracts. FAE: fruits aqueous extract, FLAE: flowers aqueous extract, LAE: leaves aqueous extract, RAE: roots aqueous extract, SAE: seeds aqueous extract, FME: fruits methanolic extract, FLME: flowers methanolic extract, LME: leaves methanolic extract, RME: roots methanolic extract and SME: seeds methanolic extract. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  (compared to  $H_2O$  as blank).

**Table 1: Polyphenol and flavonoid contents of different parts of *Capparis spinosa***

Plant parts	Aqueous extract			Methanolic extract		
	Polyphenols		Flavonoids	Polyphenols		Flavonoids
	mg GAc-Eq/g	mg Eq Rutin/g	mg Eq Quercetin/g	mg GAc-Eq/g	mg Eq Rutin/g	mg Eq Quercetin/g
Roots	$15.5 \pm 6.9^{a*}$	$1.0 \pm 0.6$	Undetectable	$9.2 \pm 2.2$	$0.3 \pm 0.2$	Undetectable
Fruits	$7.2 \pm 0.6$	$1.1 \pm 1.3$	Undetectable	$58.8 \pm 4.7^{a,b***}$	$41.9 \pm 8.6^{a,b***}$	$27.2 \pm 5.4^{a,b***}$
Leaves	$57.0 \pm 14.2^{b***}$	$11.2 \pm 0.9^{b***}$	$6.7 \pm 0.2^{b***}$	$133.6 \pm 58.8^{a,b***}$	$38.0 \pm 5.1^{a,b***}$	$24.6 \pm 3.0^{a,b***}$
Flowers	$34.1 \pm 4.6^{b***}$	$13.7 \pm 1.7^{b***}$	$8.9 \pm 1.6^{b***}$	$33.5 \pm 3.4$	$15.8 \pm 3.8^{b***}$	$9.8 \pm 2.2^{b***}$
Seeds	$35.8 \pm 2.3^{b***}$	$2.4 \pm 1.8$	$0.8 \pm 0.8$	$29.016 \pm 10.5$	$6.3 \pm 1.2^{a**, b***}$	$3.4 \pm 1.2^{a*, b**}$

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , a : comparison between aqueous and methanolic extracts, b: compared to root extract. Controls used were methanol and water which contained 0.0 mg of polyphenols and flavonoids.

The methanolic extracts inhibited the peroxidation of the linoleic acid more than aqueous extracts did for extracts from all parts of the plant (Figure 2). The methanolic extract of flower displayed the highest antioxidant activity ( $82.78 \pm 2.64\%$ ), followed by the leaf extract ( $80.94 \pm 1.57\%$ ). For the methanolic extracts the anti-lipid peroxidation activity decreased in the following order: FLME > LME > SME > FME > RME. In the aqueous extracts the order is: LAE = SAE = FLAE > FAE > RAE. Such differences in the antioxidant activity of extracts could be attributed to the content of total polyphenol compounds and flavonoids. In fact, several studies have shown a correlation between antioxidant activity and phenolic content.<sup>[21,30]</sup>

### Free radical scavenging activity using 1-Diphenyl-2-Picryl Hydrazyl (DPPH) radical

We noted that all the tested extracts have an ability to capture free radicals (Figure 3). Inhibition concentration (IC 50) values showed that methanolic extracts of leaf (LME) had the greatest potential to inhibit  $0.024 \pm 0.0005 \mu\text{g/mL}$  higher than

that of the positive control BHT  $0.0485 \pm 0.0038 \mu\text{g/mL}$ . For the methanolic extracts, the free radical scavenger activity decreased in the following order: LME > FME > SME > LME > RME. In the aqueous extracts the order was: FAE = FLAE > LAE > SAE > RAE (Figure 3). This activity could be related to the richness in polyphenols (Table 1). According to Turkmen et al.,<sup>[31]</sup> polyphenols are effective donors of hydrogen radicals to DPPH. The mechanism of the reaction between antioxidant and DPPH depends on the structural conformation of the antioxidant.<sup>[32]</sup> The crude methanolic extract merits further experiments *in vivo*.

## CONCLUSIONS

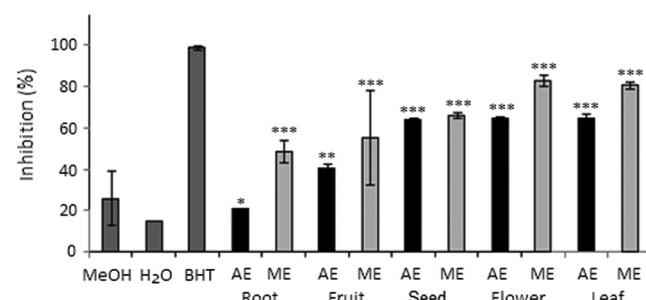
We have demonstrated here that all parts of *Capparis spinosa* have good antioxidant potential capacity and free radical scavenging. These effects may be attributed to the contents of polyphenols and may be due in majority to leaves and flowers extracts. In fact, the leaves and flowers methanolic extracts have the highest antioxidant effects compared to BHT and this is also proved by the test of beta carotene. These effects could justify the wide use of this plant in traditional medicine for the treatment of free radical related diseases such as osteoarthritis,<sup>[33]</sup> arthritis<sup>[34]</sup> and inflammation.<sup>[35]</sup>

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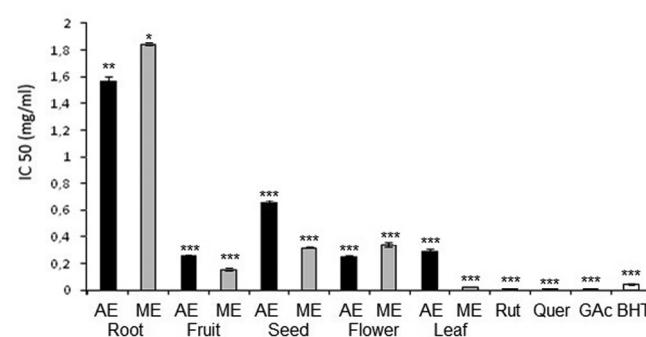
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**Figure 2.** Comparison of the antioxidant activity of the extracts of the different parts of *C. spinosa* compared to BHT and blanks (H<sub>2</sub>O and methanol) by using β-carotene-linoleic acid assay (% inhibition of the linoleic acid oxidation) after 24 hours. AE: aqueous extract, ME methanolic extract. Results are means of three different experiments. p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 (compared to H<sub>2</sub>O as blank).



**Figure 3.** DPPH free radical scavenging activity of different part of *capparis spinosa* extracts. (AE: aqueous extract; ME: methanolic extract, GAc: gallic acid, Rut: rutine, Quer: quercetin). Data are presented as IC 50 mean ± SD.

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