Pharmacogn. Mag.
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ORIGINAL ARTICLE

Intercomparative Investigation of the Total Phenols, Total Flavonoids, *In vitro* and *In vivo* Antioxidant Activities of *Capparis Cartilaginea* (Decne.) Maire and Weiller and *Capparis Ovata* Desf. from Jordan

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Submitted: 11-08-2017 Revised: 21-09-2017 Published: 28-06-2018

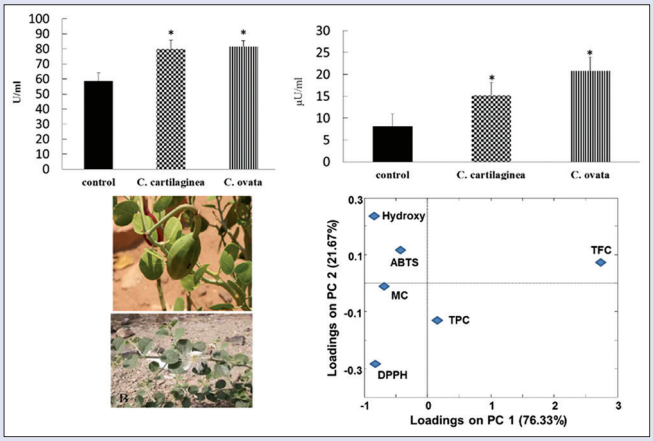
ABSTRACT

Background: Plant-derived antioxidants have been products of choice in therapeutic formulations for their potent free radical scavenging activity and mitigating and curing illnesses and diseases associated with oxidative stress. *Capparis* L. includes species with unprecedented nutritional and medicinal values, and hence offers a diverse pool of phytochemicals implicated in antioxidant activity. **Objective:** The current study was designed to assess the chemical variation, total phenols content (TPC), total flavonoids content (TFC), *in vitro* and *in vivo* antioxidant activities of different extracts obtained from *Capparis cartilaginea* and *Capparis ovata* from Jordan. **Materials and Methods:** The ethanolic extract from each species was partitioned in different solvents and the TPC, TFC, and antioxidant activity for each extract was examined. The *in vitro* antioxidant activities were tested by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino–bis (3-ethylbenzoline-6-sulfonic acid diammonium salt (ABTS) radical scavenging methods in addition to the metal ion chelating effect (MCE) and hydroxyl radical assay methods. The butanol extract was investigated for its *in vivo* effect on the activities of serum superoxide dismutase (SOD) and glutathione peroxidase (GPX) in mice (100 mg/kg, intraperitoneally for 12 days). **Results:** The butanol fraction of both plants had a dose-dependent *in vitro* antioxidant activity. The treatment of mice with the butanol extract of both species for 12 days significantly increased the activities of serum SOD and GPX. Principal component analysis indicated that DPPH, TFC, hydroxyl radical had major variability in the antioxidant activities of the two investigated *Capparis* species. Results of this study underscores enrichment of the two studied *Capparis* species with phenolics and flavonoids that could account for much of their antioxidant activities.

Key words: *Capparis cartilaginea, Capparis ovata, in vitro* antioxidant activity, *in vivo* antioxidant activity, total flavonoids, total phenols

SUMMARY

- The butanol fraction of the two Capparis species showed a dose-dependent antioxidant activity. The treatment of mice with the butanol extracts significantly increased the activities of serum SOD and GPX. Principal component analysis showed that the antioxidant assays DPPH, ABTS, MC, and hydroxyl radical differed in the degree of antioxidant activity of the butanol fractions from the two Capparis species. Results of this study underscored enrichment of Capparis species with phenolics and flavonoids that could account for much of their antioxidant activities.



Abbreviations used: C. cartilaginea: Capparis cartilaginea ; C. ovata: Capparis ovata; TPC: Total phenols content; TFC: Total flavonoids content; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid; MC: Metal Chelating effect; Hydroxy: Hydroxyl radical assay; SOD: Serum superoxide dismutase; GPX: Glutathione Peroxidase ;PC: Principal Component.

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DOI: 10.4103/pm.pm_356_17

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INTRODUCTION

Free radicals are highly unstable chemical species generated as byproducts in our bodies through metabolism, especially under stress conditions. They contain unpaired electrons and thus can easily and rapidly react with, and oxidize other molecules through giving out or accepting a single electron.[1] These highly reactive species can attack and damage macromolecules in living cells leading to a serious tissue injury or cell death.[2] Several human diseases such as aging, cancer, diabetes, liver cirrhosis, cardiovascular, and gastrointestinal disorders and many others are linked directly to the presence of free

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Cite this article as: Al-Qudah MA, Obeidat SM, Muhaidat R, Al-Trad B, Al-Jaber HI, Lahham JN. Intercomparative investigation of the total phenols, total flavonoids, *In vitro* and *In vivo* antioxidant activities of *capparis Cartilaginea* (Decne.) maire and weiller and *Capparis Ovata* Desf. from Jordan. Phcog Mag 2018;14:S154-60.

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radicals.[3,4] Antioxidants, and through their ability to cease free radical attacks and radical-mediated oxidative reactions,[4] can resolve the problems connected to the formation of free radicals in living systems. The balance between oxidation and antioxidation is believed to play an essential role in maintaining a healthy biological system. Oxidative stress, a condition of imbalance between oxidant and antioxidant mechanisms, poses risks of serious chronic and degenerative diseases, necessitating the use of antioxidant medications. Antioxidants from natural sources have been medications of choice in therapeutic formulations due to their efficient free-radical scavenging activity and safe usage compared to synthetic antioxidant supplements. Plants are recognized as valuable sources of potent antioxidants, such as carotenoids and polyphenols, that can alleviate oxidative stress and harmful effects of generated oxidants.[5]

Capparis L. (Caper), a genus of the family *Capparaceae*, has been one of the many flowering plant genera that have received special attentions in chemical, health care, and pharmacological studies.[6] The genus comprises 225 accepted species of shrubs or lianas distributed largely in warm, arid, and semi-arid habitats of the Mediterranean basin, tropical, and subtropical zones of the world.[5] Several *Capparis* species have a long history of ethnomedicinal usage, and as such are well-known and recognized for their nutritional and therapeutic values. For example, flower buds and fruits of *Capparis ovata* Desf. and *Capparis spinosa* L. are consumed pickled in various countries as sources of spicy flavor and antioxidants.[7,8] Moreover, several species are conventionally used by many cultures as herbal remedies for the treatment of many illnesses and ailments in which free radicals or oxidants are implicated to cause. These include rheumatism, anemia, arthritis, pleurisy, stomach problems, headache, toothache, earache, cough, cold, asthma, ulcers, vomiting, diabetes, fever, gout, jaundice, cholera, dysentery, smallpox, diarrhea, swelling of testicles, and cardiovascular diseases.[9-12] In the Arabia and the Middle East, an ointment is prepared conventionally from the *C. spinosa* roots and is used to relief muscle pain and disc prolapse. Previous phytochemical investigations revealed the enrichment of *Capparis* with an array of beneficial chemical constituents including glucosinolates, isothiocyanate glucosides, glycosides, flavonoids, polyphenols, phenolic acids, terpenoids, saponins, tocopherols, lignans, tannins, anthocyanins, carotenoids, sterols, lipids, and sulfides.[6,7,13-18] Of these chemical groups, the phenolics rutin and quercetin along with tocopherols and carotenoids were found to account for the antioxidant activity of the widely investigated *C. spinosa*.[6,7,16] By comparison to *C. spinosa*, little is known about the antioxidant capacities and underlying chemical groups for other medicinally and nutritionally important species of *Capparis*.

In Jordan, *Capparis* forms an important part of the vegetation, growing in a wide range of habitats and often dominates sites that are hot and arid or semi-arid.[19,20] Six species have been reported to grow wild in Jordan, namely *Capparis cartilaginea* Decne, *Capparis decidua* (Forssk.) Edgew, *Capparis leucophylla* DC., *Capparis ovata* Desf., *Capparis sicula* Duh. and *C. spinosa* L.[19,20] To the best of our knowledge, investigations on *Capparis* species from Jordanian origin for antioxidant activities and responsible phytochemical content have not been carried out. Therefore, the current study was designed to assess the chemical variation, total phenols (TPC), total flavonoids (TFC), *in vitro* and *in vivo* antioxidant activities of different extract fractions prepared from two species: *C. cartilaginea* and *C. ovata*. Most importantly and unlike prior studies which generally adopted 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method for antioxidant activity assays, we integrated four different *in vitro* methods augmented for the first time by an *in vivo* assay method to investigate and evaluate the antioxidant activities of these two different *Capparis* species.

MATERIALS AND METHODS

General

Ultraviolet (UV) spectra were measured using Biochrom WPA Wavelight II UV-visible spectrophotometer (Version 7120V1.8.0, USA). All chemicals used in this investigation were purchased from Sigma-Aldrich (Buchs, Switzerland) including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino–bis (3-ethylbenzoline-6-sulfonic acid diammonium salt (ABTS), Folin and Ciocaltea's phenol reagent, Ferrozin (3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-*p*, *p*'-disulfonic acid monosodium salt hydrate), $FeCl_2$ (VWR), NaOH, $AlCl_3$, Na_2CO_3, $NaNO_2$, H_2O_2, $FeSO_4$, and salicylic acid were also purchased from Sigma-Aldrich. $K_2S_2O_8$ was a product of Fluka, Steinheim, Germany.

Plant material and extract preparation

Fresh aerial parts of *C. cartilaginea* (voucher specimen YU/1/1001) and *C. ovata* (voucher specimen YU/1/1004) were collected during the flowering period (2015) from the Dead Sea region, Jordan. The identity of the two species and their names were confirmed using characters related to growth habits and morphological traits outlined in regional floras.[20]

Both plants were air-dried in the shade for 1 month and subjected to phytochemical analyses as previously described.[6] Afterward, they were ground to fine powders and defatted with petroleum ether in Soxhlet extractor. After this, the plant residue was extracted in the same apparatus in methanol. The obtained alcoholic gummy residue was then partitioned according to the procedure mentioned in the literature between $CHCl_3$ and H_2O (1:1). The dried chloroform residue was then subjected to partitioning between 10% aqueous methanol and hexane. The polar organic compounds were extracted from water by *n*-butanol. The different fractions obtained were then screened for their phytochemical constituents and assayed for their TPC, TFC, *in vitro* and *in vivo* antioxidant activities.

Phytochemical screening

The crude extracts obtained from *C. cartilaginea* and *C. ovata* were tested for the presence of flavonoids, alkaloids, tannins, terpenes, saponins, and glycosides according to the procedures described in the literature.[6] Qualitative results, expressed as (+) for the presence and (-) for the absence of the indicated phytochemical classes are summarized in [Table 1].

Table 1: Main secondary metabolite classes detected in the different fractions of *Capparis cartilaginea* and *Capparis ovata* growing wild in Jordan

Groups	H		A		B		W	
	C. cartilaginea	*C. ovata*	*C. cartilaginea*	*C. ovata*	*C. cartilaginea*	*C. ovata*	*C. cartilaginea*	*C. ovata*
Alkaloids	-	+	+	+	+	+	+	-
Tannins	-	-	-	+	+	+	+	+
Glycosides	-	-	-	-	-	-	-	-
Flavonoids	-	-	+	+	+	+	-	-
Sponins	+	-	+	+	+	+	-	+
Terpenes	+	-	+	+	-	-	-	-

A: H: *n*-Hexane fraction; A: Aqueous methanol fraction, B: Butanol fraction, W: Water fraction, *C. ovata*: *Capparis ovata*; *C. cartilaginea*: *Capparis cartilaginea*

Total phenols (TP) and total flavonoids (TF) content

TPs were determined using the Folin–Ciocalteu method according to the procedure described in the literature[15] with slight modifications. Briefly, 0.5 mL of each extract was treated with 2.5 mL of Folin–Ciocalteu reagent (2N) (diluted 10-fold) and 2 mL of Na_2CO_3 (75 g/L). The mixture was then allowed to stand at room temperature for 15 min, and the absorbance was recorded at 765 nm. Methanol was used as a blank. The total phenolic content in the different extracts of both species was expressed as mg/g gallic acid equivalent. All measurements were performed in triplicates [Table 2].

The TFs content in the different extracts obtained from the two *Capparis* species was determined calorimetrically using the aluminum chloride assay method.[21] Briefly, 1 mL aliquot from the stock solution (1 mg/mL) of each extract, diluted in 4 mL distilled water, was introduced into a 10 mL volumetric flask, to which 0.3 mL sodium nitrite solution (5% $NaNO_2$, w/v) was added. The resulting mixture was then allowed to stand for 5 min and 0.3 mL of aluminum chloride solution (10% $AlCl_3$, w/v) was added. The resulting solution was incubated at room temperature for another 6 min after which, 2 mL of 1.0 M NaOH solution was added and the final volume was adjusted to 10 mL with distilled water. After 15 min, the absorbance was read at 510 nm. Methanol was used as a blank. The TFs content is expressed as mg quercetin/g of dry extract. All measurements were performed in triplicates [Table 2].

Antioxidant and radical scavenging activity

The antioxidant activity of the different extracts of *C. cartilaginea* and *C. ovata* were screened using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), Metal chelating (MC) and the hydroxyl radical (hydroxy) assay methods according to the procedures mentioned in the literature.[21-31] Alpha-tocopherol, BHA, ethylenediamine tetraacetic acid and ascorbic acid were used as positive controls. The IC_{50} of the extracts and the positive controls, expressed as means ± standard deviation, are shown in [Tables 3,4]. All determinations of the IC_{50} by the three assay methods were conducted in triplicates. Preliminary results indicated that the butanol fraction obtained from both species had the highest antioxidant activity and hence was further assayed for the *in vivo* antioxidant activity.

In vivo antioxidant assay

Eighteen male adult Swiss albino mice, 55–60 days old and weighing approximately 30 g each, were preserved in the animal house at Yarmouk University under 12/12 h light/dark photoperiod and 21°C–23°C temperature regime. Standard pellet food and water were provided *ad libitum*. Mice were divided into three groups (n = 6 each): control, *C. cartilaginea*, and *C. ovata* butanol fractions treated groups (100 mg/kg, intraperitoneally for 12 days). At the end of the experiments, the animals were weighed and anesthetized by ether, and blood was collected through cardiac puncture. Following blood clotting and centrifugation, serum was decanted and stored at −80°C until biochemical analyses. Activities of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX) were assayed in serum using standard colorimetric assay kits (AAT Bioquest and BioVision, respectively, Sunnyvale, CA, USA) according to the manufacturers instructions.

Analyses of correlations among the evaluated parameters and statistical analysis

Principal component analysis (PCA) was used in this study to establish the relationships among the different variables. The analysis was performed using PLS Toolbox 4.0 (Eigenvector Research, Inc, WA and USA) under MATLAB 7.0.4 (MathWorks, MA, USA). Statistical analyses of data were performed using the SPSS software version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). P values were determined using one-way ANOVA followed by LSD or Student's t-test, as appropriate. Differences were considered statistically significant if $P < 0.05$. Data were expressed as the means ± standard error of the mean.

RESULTS AND DISCUSSION

The results of the qualitative phytochemical screening of the different fractions of *C. cartilaginea* and *C. ovata* are summarized in Table 1. Alkaloids were detected in the aqueous methanol and the butanol fractions of *C. cartilaginea* and *C. ovata*, in the water fraction of *C. cartilaginea* and the hexane fraction of *C. ovata*. In addition, the aqueous methanol and butanol fractions of both plants showed positive tests for the presence of flavonoids and saponins. Saponins were also detected in the water fraction of *C. ovata* and the hexane fraction of *C. cartilaginea*. Moreover, tannins were detected in the aqueous methanol, butanol and water fractions of *C. ovata*, and only in the butanol and water fractions of *C. cartilaginea*. Terpenes, on the other hand, were detected in the aqueous methanol of both plants and in the hexane fraction obtained from *C. cartilaginea*. All four fractions obtained from the two investigated *Capparis* species gave negative results for glycosides.

Our screening results clearly indicated the presence of large amounts of phenolic compounds, particularly flavonoids. For this reason, the TP and TF contents in both species were investigated [Table 2]. It was found that the total phenolic content (expressed as mg/g gallic acid) varied in the different extracts, ranging between 21.00 and 233.50 mg/g gallic acid for *C. cartilaginea* and 16.17–293.50 mg/g gallic acid for *C. ovata*. Among the different extracts tested, the butanol fractions from both species showed the highest phenolic content [Table 3]. This was augmented by the high flavonoids content (measured as mg quercetin/g of dry extract)

Table 2: Total phenolic content and total flavonoids of all extracts of *Capparis cartilaginea* and *Capparis ovata* from Jordan

Extracts	Total phenols (mg/g gallic acid)*		Total flavonoids (mg/g quercetin)*	
	C. cartilaginea	*C. ovata*	*C. cartilaginea*	*C. ovata*
Hexane	21.00±1.00	16.17±1.26	-	-
Aqueous methanol	177.2±3.93	172.83±4.31	779.94±4.56	590.74±3.87
Butanol	233.50±1.18	293.50±5.07	966.61±3.40	920.14±4.34
Water	74.50±1.80	66.17±2.57	90.33±5.65	70.37±2.00

*Mean values followed by different superscript are significantly different (P<0.05). Values expressed are means±SD of three parallel measurements. SD: Standard deviation

Table 3: Antioxidant activities of extracts and standards by using DPPH and ABTS methods of *Capparis cartilaginea* and *Capparis ovata* from Jordan

Extracts	DPPH IC_{50} (mg/mL)		ABTS IC_{50} (mg/mL)	
	C. cartilaginea	*C. ovata*	*C. cartilaginea*	*C. ovata*
Butanol	0.15±0.001	0.06±0.002	0.09±0.01	0.04±0.003
Aqueous methanol	0.89±0.20	0.19±0.07	0.68±0.08	0.11±0.003
Water	3.06±1.42	0.22±0.03	1.13±0.09	0.33±0.017
Hexane	ND	ND	ND	ND
α-tocopherol	$0.019±2.36×10^{-3}$		$0.054±2.86×10^{-3}$	
Ascorbic acid	$7.22×10^{-5}±2.30×10^{-6}$		0.010±0.00	

Mean values are significantly different (P<0.05). Values expressed are means±SD of three parallel measurements. ND: Not determined; SD: Standard deviation

detected in the butanol fraction of *C. cartilaginea* (966.61 ± 3.40) and *C. ovata* (920.14 ± 4.34).

The different classes of secondary metabolites detected in all fractions of *C. cartilaginea* and *C. ovata* are well known for their important pharmaceutical activities, especially antioxidant properties.[32-36] In the literature, the conventional method of DPPH assay was commonly used for evaluating the antioxidant capacities for various plant materials *in vitro*.[37-40] In the current study, by comparison, the antioxidant activities for the different extract fractions from the two *Capparis* species were assessed using four different *in vitro* methods including the DPPH, ABTS scavenging, MC, and hydrogen peroxide scavenging assay methods supported by an *in vivo* study. In this study, a series of concentrations ranging from 0.005 to 1.0 mg/mL were prepared from each fraction and screened for antioxidant and radical scavenging activities using the aforementioned methods [Tables 3 and 4].

Results clearly indicated that the DPPH and ABTS radical scavenging activities were concentration dependent [Table 3 and Figures 1, 2]. For both plant species studied here, the order of radical scavenging power for the different extract fractions as measured by both assays was as follows: Butanol > aqueous methanol > water. However, hexane did not show any activity. Interestingly, the radical scavenging power of the crude butanol fraction from *C. ovata* (IC_{50}: DPPH: 0.06 ± 0.002; ABTS: 0.04 ± 0.003) demonstrated a 2.5-fold higher capacity compared to the butanol fraction from *C. cartilaginea* (IC_{50}: DPPH: 0.15 ± 0.001; ABTS: 0.09 ± 0.01) [Table 3]. The stronger scavenging activity of the butanol fractions from *C. ovata* and *C. cartilaginea* could be due to its relatively higher content of phenol and flavonoid compounds, which are known for their potent antioxidant capacity.

The chelating activity of crude extracts from *C. cartilaginea* and *C. ovata* were also investigated using the MC method and results are presented in Figure 3. It was shown that the chelating activity increased in a dose-dependent manner. Based on the IC_{50} values, the butanol fraction of *C. cartilaginea* (0.02 ± 0.005 mg/mL) and aqueous methanol fraction of *C. ovata* (0.09 ± 0.006 mg/mL) had the highest chelating activity [Table 4]. This could also be attributed to the high content of phenols and flavonoids in these two fractions.

Hydroxyl radicals are known to be highly reactive species toward proteins, lipids, DNA and are known to be severely harmful for cell survival

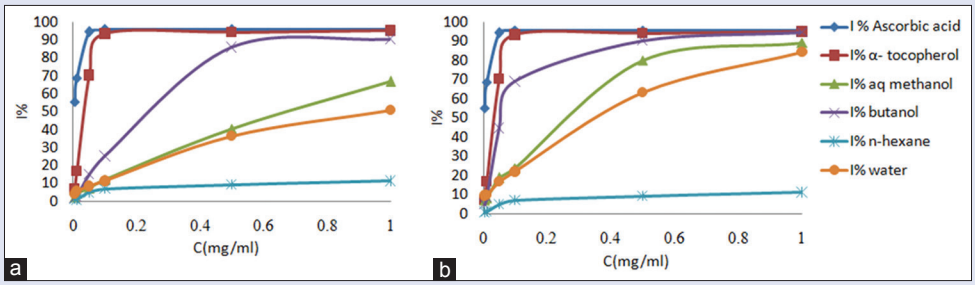

Figure 1: Antioxidant activities of extracts and standards by using DPPH method of *C. cartilaginea* (a) and *C. ovata* (b), Results are expressed as mean ± standard deviation (*n* = 3)

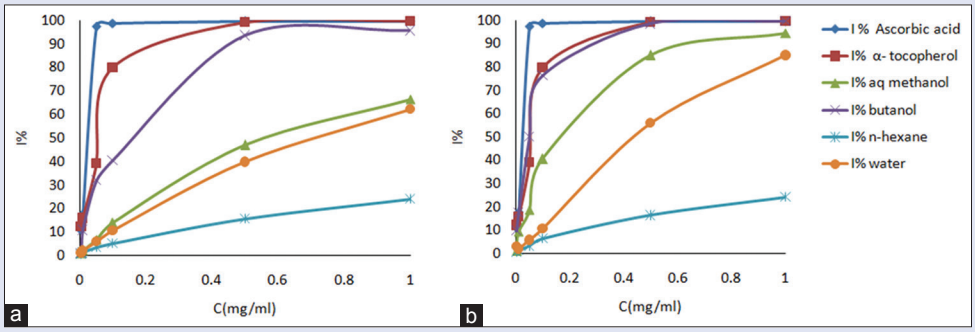

Figure 2: Antioxidant activity of the of the extract fractions of C. cartilaginea (a) and C. ovata (b)) and positive controls (ascorbic acid and α-Tocopherol) on $ABTS^{+\cdot}$ assay. I%: Inhibition in ABTS values. Results are expressed as mean±SD (*n*=3)

Table 4: Metal chelating effect and hydroxyl radical assay of the four different extracts of *Capparis cartilaginea* and *Capparis ovata*

Extracts	Ferrous chelating effect IC_{50} (mg/mL)*		Hydroxyl radical assay IC_{50} (mg/mL)*	
	C. cartilaginea	*C. ovata*	*C. cartilaginea*	*C. ovata*
Butanol	0.02±0.005	0.15±0.019	0.04±0.01	0.05±0.001
Aqueous methanol	0.34±0.010	0.09±0.006	0.10±0.09	$4.91\times10^{-3}\pm5.40\times10^{-4}$
Water	0.30±0.070	0.17±0.09	0.12±0.006	729.61±6.52
Hexane	12.48±0.25	16.76±6.56	9.80±2.40[d]	ND
EDTA	$5.58\times10^{-4}\pm3.31\times10^{-5}$		-	
Ascorbic acid	0.42±0.08		0.11 ± 2.13×10-3	

*Mean values are significantly different ($P<0.05$). ND: Not determined; EDTA: Ethylenediamine tetraacetic acid

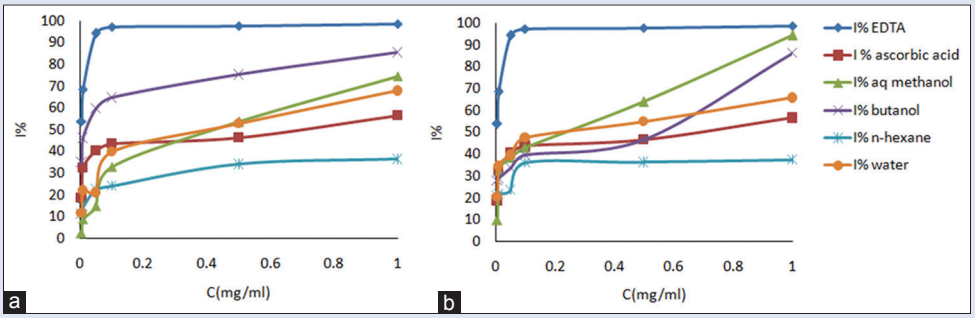

Figure 3: Metal chelating effect assay of the different fractions obtained from *C. cartilaginea* (a) and *C. ovata* (b) and positive controls (EDTA and ascorbic acid); I%: Metal chelating activity, Results are expressed as mean ± standard devition ($n = 3$)

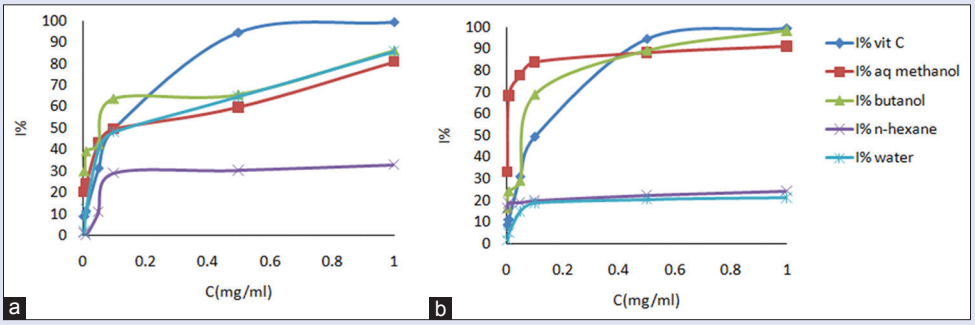

Figure 4: Hydroxyl radical assay of the different fractions obtained from *C. cartilaginea* (a) and *C. ovata* (b) and positive control (ascorbic acid); I%: Hydroxyl radical scavenging value

when overproduced.[30] Removal of these radicals is thus so important for living systems to maintain the redox homeostasis. Figure 4 shows the scavenging activities of the extracts obtained from *C. cartilaginea* and *C. ovata*. Our results revealed that the hydroxyl radical scavenging activity was also concentration dependent. The butanol fraction of *C. cartilaginea* and the aqueous methanol one of *C. ovata* were the most active fractions [Table 4].

In vivo antioxidant activity

Since the butanol fractions from both *Capparis* species had the highest total phenolic and flavonoids content, and in most cases demonstrated the highest antioxidant and radical scavenging activities *in vitro*, it was preferably assessed for antioxidant activity assay *in vivo*. To meet this goal, the activities of two key antioxidant enzymes (SOD, GPX) were assessed. It was found that activities of both enzymes increased significantly in the butanol extract treated groups compared to the control group. This provides robust evidence that the butanol extract of both *C. cartilaginea* and *C. ovata* is a potent source of natural antioxidants.

Interestingly, quantitative differences between our study of the antioxidant and radical scavenging activities as determined by the ABTS method for aqueous and methanolic extracts of *C. cartilaginea* and findings of a previously published study were found.[41] Alali *et al.* reported the total antioxidant capacity of the aqueous and methanolic extracts of *C. cartilaginea* was 91.8 ± 4.6 and 81.1 ± 2.9, respectively. In our study, by comparison, the corresponding antioxidant capacities of the aqueous and methanolic extracts from the same species were 62.2 ± 2.04 and 66.55 ± 0.91, respectively. The variations between these two studies could be due to differences in plant parts used in the extraction process and methods of the extraction [Figures 5 and 6].[42]

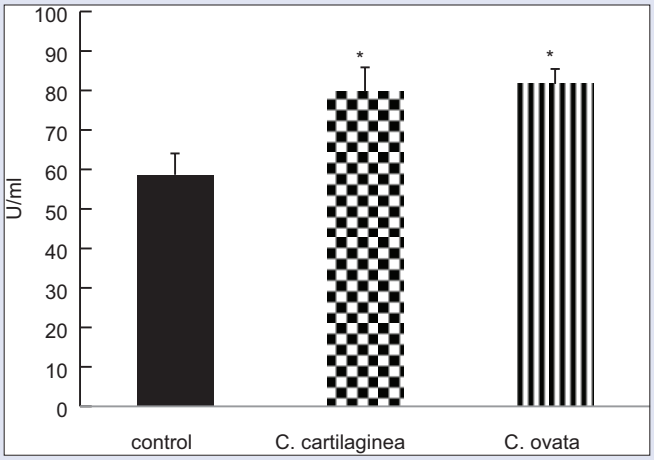

Figure 5: Serum serum superoxide dismutase levels of mice treated intraperitoneally for 12 days with 100 mg/kg of *Capparis cartilaginea* and *Capparis ovata* butanol fraction. Data represent the mean ± standard error of mean. *$P < 0.05$ in compared to the control group

Principal component analysis

PCA was used to establish the relationships between the TPC and TFC in the butanol fraction of these two plants and their DPPH, ABTS, MC, and hydroxyl radical scavenging activities. In addition, this analysis is used also to uncover the contribution of each method for the total antioxidant activity. Our PCA analysis revealed a strong positive correlation between the TFC and the antioxidant activity, whereas TPC showed a less positive correlation to the PC1 [Figure 7]. However, on the other hand, Hydroxyl, ABTS, and DPPH radical variables contributed negatively to PC1. The

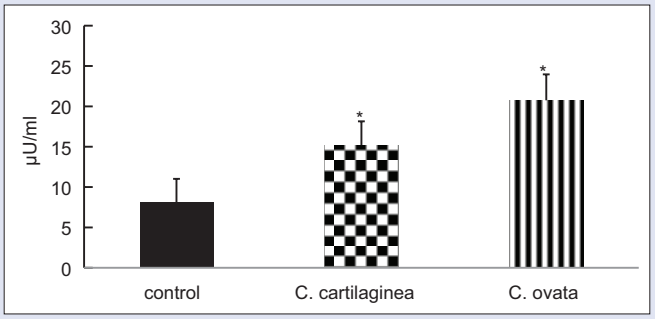

Figure 6: Serum glutathione peroxidase levels of mice treated intraperitoneally for 12 days with 100 mg/kg of *Capparis cartilaginea* and *Capparis ovata* butanol extract. Data represent the mean ± standard error of mean. *$P < 0.05$ in compared to the control group

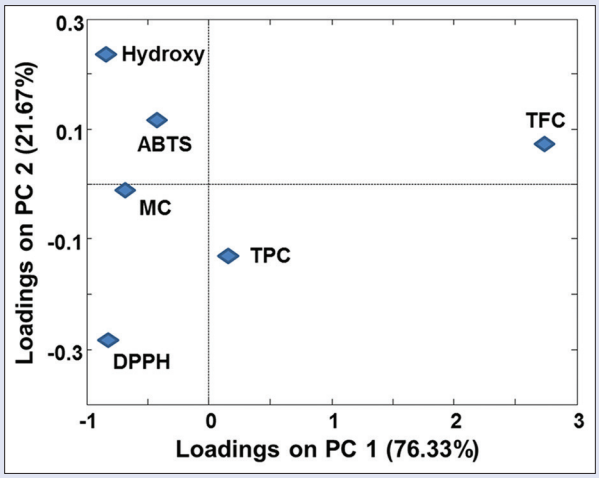

Figure 7: Principal component analysis (loadings plot) of DPPH, MC, TPC, TFC, ABTS and Hydroxyl radical assay (Hydroxy)

main positive loadings of PC2 were the hydroxyl radical assay and negatively loaded on DPPH while the rest of variables had no or very little correlation on PC2.

Furthermore, it can be seen in Figure 7 that TFC and TPC are positively correlated to each other and negatively correlated to the rest of variables while hydroxyl radical variable in negatively correlated to the DPPH radical and very limited correlation to the rest of variables.

Acknowledgements

The authors would like to thank the Deanship of Scientific Research and Graduate Studies of Yarmouk University, Irbid, Jordan, (grants number 2/2013). We also thank Fida E. Allahham and Alaa Al-Masri for technical help.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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