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تقييم الخواص المضادة للأكسدة لثمرة نبات الكبر (كابارس سبنوزا) من البحرين

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الملخص:

نبات الكَبَر (القَبَّار أو الشَفَلَّح أو الأَصَف) (*Capparisspinosa*) هي شجيرة تنتمي للفصيلة القبارية (Capparidaceae) أصلها ومؤلها الرئيسي بلدان حوض البحر الأبيض المتوسط، وتنمو بشكل جيد في بيئات مختلفة تحت أشعة الشمس الشديدة وفي درجات الحرارة الجافة، كما تتحمل درجات الحرارة المنخفضة. وفي هذه الدراسة تم تقييم الخواص المضادة للأكسدة لعينات من ثمرة نبات الكبر جمعت من بيئات متباينة، باستخدام عدة طرق وتشمل FRAP و DPPH و ABTS، كما تم تقدير نسبة المركبات الفينولية الكلية والمركبات الفلافونويدية الكلية والمركبات الكاروتينة الكلية. وقد تم احتساب القدرة المضادة للأكسدة على أساس المكافئ للملي مول للترولوكس (Trolox) لكل كجم من الوزن الطازج، حيث وجد أن متوسطه الحسابي بطريقة الـ FRAP (9.059±1.450) ، وطريقة الـDPPH (6.131±0.607) وطريقة الـABTS (8.127±2.017). أما القدرة على كسح الشوارد الحرة وتحييدها فقد بلغ متوسطها الحسابي 7.19% ± 37.67% بطريقة الـ DPPH و 7.76% ± 31.29% بطريقة الـ ABTS. وكشفت دراسات التجزئة على أن أعلى قدرة مضادة للأكسدة ومضادة للشوارد الحرة موجودة في البذور بالمقارنة مع لب الثمرة، وأن هذه القدرات مرتبطة أساسا بالمكونات الذائبة في الماء، كما أنها مرتبطة بشدة مع نسبة الفينولات الحرة والفلافونات والكاروتينات الكلية. وتساهم نتائج هذه الدراسة في زيادة المعرفة العلمية بهذه الفاكهة البرية التي يجب أن يعاد النظر فيها لما تحتويه من مكونات مهمة ذات خصائص مضادة للأكسدة.

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ORIGINAL ARTICLE

Assessment of the antioxidant properties of the caper fruit (*Capparis spinosa* L.) from Bahrain

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Abstract *Capparis spinosa*, a wild plant of the family *Capparidaceae*, is said to be native to the Mediterranean basin and found in many parts. This shrub can grow very well on dry heat and under intense sunlight and can tolerate low temperature. Caper berries from different locations of contrasting habitats were evaluated for their antioxidant capacities using several methods, including reduction of ferrictripiridyltriazine [Fe(III)-TPTZ] (FRAP assay), 1,1-diphenyl-2-picrylhydrazyl radical scavenging (DPPH assay), and [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] scavenging (ABTS assay). Total free phenolics, total flavonoids, and total carotenoids were also examined. The mean mmol Trolox equivalent/kg fresh weight for FRAP was 9.059 ± 1.450, DPPH 6.131 ± 0.607, and ABTS 8.127 ± 2,017. Mean % DPPH scavenging activity was 37.67% ± 7.19 and mean % ABTS scavenging activity was 31.29% ± 7.76. Partitioning studies revealed a higher antioxidant–antiradical capacity in the seed as compared to the flesh, and these capacities were associated more with hydrophobic and not lipophilic constituents. Antioxidant capacities strongly correlated with the total free phenolics, total flavonoids, and total carotenoids. Findings of this study contribute to the recent increase in scientific interest of a wild fruit which should be re-considered for its antioxidant constituencies and characteristics.
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1. Introduction

The genus *Capparis* belongs to the *Capparidaceae* family (Inocencio et al., 2006; Tlili et al., 2011). Wild species of *Capparis* are found in countries surrounding the Mediterranean basin extending to the Great Sahara in North Africa and the dry regions of Western and Central Asia. There are many important caper species of which *Capparis spinosa* is the most important one. Most of these species are used as gourmet food in some countries (Tlili et al., 2011). In Bahrain, Capparis genus is represented by *C. spinosa* which grows in sandy or gravelly soils in cultivated and uncultivated lands, as well as on roadsides (Phillips, 1988). Modern cultivation techniques have been applied to make it a commercial commodity (Suleiman et al., 2009).

Due to its diverse economic, ecological, and medicinal attributes, *C. spinosa* ranks among the most important species of the genus Capparis. Tlili et al. (2010) recently reviewed the ethnopharmacology, phytochemical and pharmacological properties of the caper. It has been described in many parts around the world for the treatment of many ailments (Tlili et al., 2011). According to Sher and Alyemeni (2010), many

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parts of caper are still being used as drugs and traditional healers in Saudi Arabia describe oral administration of dried fruits of *C. spinosa* with water to treat hypertension and diabetic complications. Hot-water extract of *C. spinosa* prevents lead acetate-induced lipid oxidation in rats (Al-Soqeer, 2011).

Fruits of *C. spinosa*, also known as caper berries, are ellipsoid, ovoid to pear-shaped. In Mediterranean countries, pickled bud is a commercial commodity (Sozzi, 2008), whereas in Eastern India, fresh raw caper berries are consumed as an appetizer. In Bahrain, fresh caper berries are still eaten by the elderly. In Saudi Arabia, different parts are used as vegetable and flavoring agent (Sher and Alyemeni, 2010). Fruit splits when ripe to reveal its content which includes the red flesh and seeds. The exposed content attracts insects and birds.

From nutritional point of view, berries of *C. spinosa* possess carbohydrate content (5%), dietary fibers (3%), protein (2%), and lipids (0.9%). It contains moderate content of vitamin C (4 mg/100 g fw) (USDA National Nutrient Database, 2010). *C. spinosa*, however, is widely appreciated for many claimed and proven pharmacological activities (Tlili et al., 2011). Besides, current trends in dietary guidelines place more emphasis on the phytochemical attributes. As such, seeds of caper berries are a rich source of unsaturated lipids (Matthäus and Özcan, 2005), tocopherols, sterols, and carotenoids (Tlili et al., 2009). Furthermore, fruit pulp is also rich in phenolic compounds such as rutin and quercetin (Aliyazicioglu et al., 2013; Zia-Ul-Haq et al., 2011). These phenolics, along with vitamin C, tocopherols, and carotenoids, are responsible for the antioxidant activity of plant materials (Zia-Ul-Haq et al., 2011).

Antioxidant activity of various parts of *C. spinosa* was studied by many researchers from different countries around the world (Bonita et al., 2002; Germanò et al., 2002; Tesoriere et al., 2007; Tlili et al., 2010, 2011; Yue-lan et al., 2010; Aliyazicioglu et al., 2013). Fresh and pickled flower buds were the most studied parts of the plant. According to Tlili et al. (2010), phenolic content of fresh buds and leaves from *C. spinosa* exceeded those of many commonly known rich sources of these compounds such as bilberry and raspberry. Despite these studies, little is known about the antioxidant properties of the fruit of *C. spinosa*, This study aimed to evaluate *in vitro*, the antioxidant activity and antioxidant constituents in ripe caper berries collected from different locations in the Kingdom of Bahrain; to study the hydrophilic/lipophilic partitioning of the activity, as well as to study the distribution of this activity between the flesh and seed of the fruit.

2. Materials and methods

2.1. Chemicals, reagents, and equipments

L-ascorbic acid was from BDH (UK). 2,20-Diphenyl-1-picryl hydrazyl (DPPH), gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-azinobis (3-ethylbenzothiazolin) 6-sulfonic acid (ABTS), Rutin, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), were from Sigma–Aldrich (St. Louis, USA). All other reagents and chemicals were of analytical grade. Spectrophotometric measurements were performed using a Spectronic 20 Genesys (UK). Centrifugation was performed using Beckman high speed CS-6 (Beckman, USA).

2.2. Sample locations and collection

For characterization of the antioxidant activity and constituency of caper fruits, ripped berries were collected from different shrubs during the months from September to November 2010 from several locations in the main island of the Kingdom of Bahrain. Fruits were handpicked, transported to the laboratory, kept in the refrigerator, and analyzed within three days of collection. To investigate the seasonal variation using the FRAP assay, samples were collected as described above during six successive seasons (2002–2012).

2.3. Sample preparation and extraction

Fruits were rinsed under running tap water and dried-plotted. A total of twenty ripe fruits were randomly selected. Each was cut into 2 halves, one half singly tested, and the second was used to prepare the pooled (composite) sample. The whole edible content, which included the flesh and the seeds, was examined. In another set of experiments, seeds were hand-picked and separated from the flesh, and each part was separately examined.

Methanolic extraction was prepared by adding 5 ml of 80% methanol to 1 g of the sample and grinding using pestle and mortar until a uniform mixture was obtained. The mixture was transferred into a centrifugation tube and the pestle was rinsed with 80% methanol and the final volume was brought to 10 ml. The mixture was further homogenized using a Waring homogenizer for one min at high speed. Pestle and mortar were used to ensure full disintegration of seeds, which was not practically possible by using the homogenizer alone. This one-tenth homogenate was centrifuged at 3500 rpm for 15 min.

A composite sample was prepared by pooling the other halves of the fruits. The pooled sample was extracted as above. For the partitioning study of hydrophilic and lipophilic antioxidant capacities, the same extraction procedure was used, except that a 10-g sample was first homogenized and extracted with 25 ml of 80% methanol, followed by adding 25 ml of hexane-dichloromethane (2:1) to the homogenate, grinding, and phase separation by centrifugation at 8000 rpm for 30 min. The aqueous methanolic phase was re-extracted with the same organic solvent, centrifuged and the organic phases were combined and the volume was adjusted to 50 ml. Each phase was separately analyzed.

2.4. Antioxidant activity

2.4.1. Ferric reducing ability of plasma (FRAP) assay

The manual FRAP assay used was essentially as described by Benzie and Strain (1996). To a 3 ml of FRAP reagent, an aliquot (50 μl) was added. Absorbance at 593 nm was measured before the addition of the sample or the standard and after 5 min incubation at room temperature. The standard assay used 50 μl of the sample (5 mg/ml fresh weight, FW), whereas, for dose/response experiments, the aliquot volumes varied from 10 to 100 μl. Ascorbic acid and Trolox were both used to prepare standard curves in the range of 25–1000 μM. The FRAP values were calculated using these standard curves and expressed as mmol AAE/kg FW for ascorbic acid, or mmol TEAC/kg for FW Trolox. All determinations were carried out in triplicate.

2.4.2. DPPH assay

The method described by Molyneux (2004) was used to measure the free DPPH radical scavenging activity with some modifications. An aliquot (50 μl, 5 mg/ml) was added to 2 ml of 100 μM methanolic solution. Absorbance was measured at 517 nm at 0 and 15 min incubation at room temperature. Percent inhibition activity was calculated:

$$\%\text{inhibition} = ((A_{DPPH} - A_{sample})/A_{DPPH}) \times 100\%$$

where A_{DPPH} – absorbance of DPPH$^{\bullet}$ solution in the absence of sample or standard, and A_{sample} – absorbance of a DPPH solution with a tested sample or positive control. The antiradical activity (EC_{50}) defined as the concentration of the sample resulting in 50% inhibition was calculated from a dose–response plot, in which % inhibition against extract (in volumes from 5 to 50 μl) concentration was plotted. Tests were carried out in triplicate.

2.4.3. ABTS assay

This method was essentially as described by (Re et al., 1999) with some modifications. To generate the $ABTS^{\bullet+}$, a solution of ABTS (7 mM), prepared in water, was reacted with potassium persulfate solution (2.45 mM final concentration) in brown bottle in the dark at room temperature for 16–20 h. The working reagent was prepared by diluting the above solution with Tris–HCl (pH 7.4, 10 mM) to give an absorbance of 0.70 ± 0.02 at 734 nm. In the standard assay, an aliquot of 10 μl extract, containing 1 mg/ml fw, was added to 1 ml of $ABTS^{\bullet+}$ solution. Various volumes of samples and standard (0 to 30 μl) were used in experiments conducted to calculate EC_{50} and to generate the standard curve. Absorbance at 734 nm was measured at zero and 6 min. Tris buffer was used as a solvent blank. Trolox was used as a positive control and to generate the standard curve to calculate the Trolox equivalent antioxidant capacity. $ABTS^{\bullet+}$ Scavenging % was calculated as follows:

$$\%\text{ABTS scavenging} = (\text{A723sample}/\Delta\text{A723control}) \times 100$$

Scavenging % was plotted as a function of mg fresh weight contained in the sample in order to obtain the mg required to reduce absorbance by 50% (EC_{50}). All determinations were carried out in triplicate.

2.5. Determination of antioxidant constituents

2.5.1. Total free phenolics

The Folin–Ciocalteau (FC) micro method was used to measure total phenolics as described by Waterhouse (2002). Briefly, an aliquot of 20 μl of the appropriately diluted extract was added to 1580 μl water. Concentrated FC phenol reagent (100 μl) was added. After 5 min incubation at room temperature, 300 μl of sodium carbonate (20%) was added, mixed, and the reaction mixture was incubated at 40 °C for 30 min. Absorbance was measured at 765 nm. Gallic acid, in the range of 0.05 to 1 mg/ml was used to generate the standard curve, and total phenolic content was expressed as gallic acid equivalent (GAE). Tests were carried out in duplicate.

2.5.2. Total flavonoids

Quantification of flavonoids was carried out by the aluminum chloride colorimetric method as described by Zhishen et al. (1999). An aliquot of the methanolic extract of the caber-berry was brought to 1 ml with water and 60 μl of 5% $NaNO_2$ solution (5%) was added. Following 5 min incubation at room temperature, 60 μl of $AlCl_3$ solution (10%) was added. After 6 min, 400 μl of 1 M NaOH was added and the volume was brought to 2 ml with water. Absorbance was measured at 510 nm against the blank. Rutin was used in the range of 40–400 μg/ml to prepare the calibration curve, and total flavonoid content is expressed as mg equivalents of rutin (mg RE). All determinations were carried out in duplicate.

2.5.3. Total carotenoids and lycopene

Total carotenoids were determined by a method described by Wang et al. (2005). One-tenth homogenate in 80% methanol (10 ml) was prepared as described above. This homogenate was successively extracted by addition of 5 ml hexane until the solvent became colorless. The volume was adjusted to 25 ml, appropriately diluted and absorbance was measured at 450, 645, and 663 nm. The following formulae were used to calculated total carotenoids, lycopene, and chlorophylls:

$$\text{Total carotenoids}(\mu g/ml) = 4 \times A_{450nm}$$
$$\text{Chlorophyll}(\mu/ml) = (20.2 \times OD_{645nm}) + (8.2 \times OD_{663nm})$$

Under experimental conditions employed, chlorophyll was not detected, hence, it will be not be further mentioned.

2.5.4. Statistical analysis

Excel (Microsoft, USA) and SPSS (version 15, USA) were used for statistical analysis. Results are given as means and standard deviation (SD). Pearson's correlation was used to evaluate the relationship between activities and constituents.

3. Results and discussion

3.1. Antioxidant activity

Table 1 shows the antioxidant/antiradical activity of the methanolic extract prepared from *C. spinosa* fruits collected from different locations in Bahrain. Fruits from these locations showed variable but high antioxidant activities in the three assays tested (FRAP, DPPH, and ABTS). Large variations in the antioxidant activities were observed when each berry was singly tested, and variations were statistically significant ($p = 0.000$). Variations in antioxidant activity between and within each food group are well documented. Antioxidant activity exhibited a dose dependent activity in all assays used.

3.1.1. FRAP assay

Table 1 shows the antioxidant activity and FRAP value of the methanolic extract of fresh fruit of *C. spinosa*. The overall FRAP value averaged 9.059 ± 1.450 mmol TEAC/kg FW, and ranged from to 6.023 to 11.12 mmol TEAC/kg FW. FRAP values expressed as mmol AAE/kg fw were similar to those expressed as TEAC, 8.493 (±1.360), which is in agreement with observations of original developer of the FRAP values, where ascorbic acid and Trolox have similar relative activity (Benzie and Strain, 1996).

Recently, the FRAP values of *C. spinosa* were reported by several investigators. Bhoyar et al. (2011), reported that FRAP values of dried samples of arid desert of trans-Himalayas were

Table 1 Means (± SD) of FRAP value, DPPH and ABTS radical scavenging activity of *C. spinosa* fruits. Values are expressed as mmol TEAC/kg fresh weight (FW).

	FRAP	DPPH	ABTS
Standard assay[a]			
mmol Trolox/kg FW	9.059 (1.450)	6.131 (0.607)	8.127 (2.017)
Radicals scavenging activity			
% inhibition[b]	–	37.673 (7.192)	31.289 (7.636)
EC_{50} (mg FW)[c]	–	3.95 (0.63)	0.55 (0.031)
Fractionation experiment[d] (TEAC mmol /kg FW)			
Hydrophilic fraction	12.777 (0.590)	6.445 (0.083)	9.821 (3.057)
Lipophilic fraction	0.48 (0.188)	2.859 (0.127)	2.186 (0.361)
Partitioning experiment			
(TEAC mmol/kg FW)			
Flesh	7.92 (0.151)	2.215 (0.106)	6.392 (0.478)
Seed	17.76 (0.038)	6.628 (0.261)	13.214 (0.321)
Whole	14. 390 (0.085)	5.537 (0.470)	9.424 (0.153)

[a] Data of standard assay are shown as means ± SD from twenty fruits extracted individually and tested in triplicate. An aliquot of 50 µl (5 mg/ml FW) was used in the standard assay.
[b] % inhibition of scavenging activity was performed using the standard assay; 50 µl of 5 and 1 mg/ml (FW) in DPPH and ABTS assays, respectively.
[c] Scavenging activity (EC_{50}) was calculated from dose–response plots.
[d] Data of fractionation and partitioning experiments were from pooled samples tested in triplicate.

expressed as percentage of the reference antioxidant (butylated hydroxytoluene; BHT). Aliyazicioglu et al. (2013) reported a FRAP value of 145 µmol Trolox/100 g DW for dried *C. spinosa* from Turkey. Zia-Ul-Haq et al. (2011) using extracted dried samples of a related species, *Capparis deciduas*, presented a comparatively very high FRAP value of 2388 µmol TE/100 g DW. All these reported values cannot directly be compared with values given in this study since the values were either for dried samples or expressed differently.

FRAP assay was used to evaluate the total antioxidant content of 3100 foods of various categories used worldwide (Carlsen et al., 2010). FRAP values, expressed as mmol/100 g, of common fresh fruits were comparable to fresh caper-berries (i.e. apple, 0.4; orange, 0.9; papaya, 0.6; pomegranate, 1.8; and strawberries, 2.1, respectively). Furthermore, Jabońska-Ryś et al. (2009), recently reported FRAP value of several Polish wild edible fruits, ranging from as low as 11 mmol AAE/100 g FW (wild strawberry and rowan) to as high as 64 mmol AAE/100 g FW (dog rose). Wild Italian blueberries showed FRAP value of 52–57 mmol/kg FW (Giovanelli and Buratti, 2009).

FRAP assay uses an oxidation/reduction reaction to measure the ability of a sample (an antioxidant) to reduce TPTZ-Fe^{3+}complex to TPTZ-Fe^{2+}(Zia-Ul-Haq et al., 2013). However, it measures antioxidants with a reduction potential below the reduction potential of the Fe^{3+}/Fe^{2+} couple (0.77 V). Thus antioxidant with a reduction potential greater than the Fe^{3+}/Fe^{2+} couple, such as glutathione and small molecular weight thiols and sulfur containing compounds, will not be measured. In fact, caperberries are a good source of these compounds (Afsharypuor et al., 1998). Romeo et al. (2007) reported that pickled caper contained 8.4% sulfur compounds, of which methyl-isothiocyanate and benzyl-isothiocyanate where the major. Hence, FRAP values reported here may be an underestimate of the actual value. The content of thiols and sulfur containing compounds of the fresh tested fruits were not investigated in this study. To the author knowledge, FRAP values of fresh caper-berries were not reported before.

3.1.2. DPPH radical scavenging activity

% DPP radical scavenging activity is shown in Table 1. At a concentration of 5 mg/ml fw, caper fruits possessed varied DPPH radical inhibition activity ranging from 22% to 45% with an overall average of 37.8%. Amount needed to achieve 50% DPPH inhibition (EC_{50}) from several composite samples averaged 3.95 ± 0.63 and ranged between 2.1 to 5.8 mg. EC_{50} of Trolox was 0.0123 mg/ml. When the DPPH scavenging activity is expressed as mmol TEAC/kg FW, it ranged from 4.02 to 8.50, and the average value was 6.131 ± 0.607 mmol TEAC/kg FW.

Several authors reported DPPH scavenging activity of different parts of *C. spinosa*. Germanò et al. (2002) reported that methanolic extract of caper buds showed strong activities in DPPH assay. According to Bonita et al. (2002) the scavenging effect of lyophilized extract of buds of *C. spinosa* was concentration-dependent giving an EC_{50} value of 68.36 mg/ml. In contrast, much lower concentration (0.32 mg/ml) was needed to give IC_{50} by methanolic extracts of fruits of *C. spinosa* from Turkey (Aliyazicioglu et al., 2013). Yang et al. (2010) isolated several compounds from caper fruits with a strong DPPH scavenging activity. Direct comparison of data from the current study with those reported in the literature is difficult since different parts and expression units were used.

3.1.3. ABTS radical scavenging activity

As with the two other assays, methanolic extracts of fresh fruits at concentration of one mg/ml fw showed variable percent ABTS radical scavenging activity ranging from 23.1% to 44.9% with an overall average of 31.29 ± 7.64%, and the EC_{50} calculated from two pooled samples averaged 0.55 ± 0.31 and ranged 0.51 and 0.60 mg/ml FW (Table 1). In the standard assay, when the radical activity is expressed as mmol TEAC/kg fw, it ranged from 5.99 to 11.87 and averaged 8.127 ± 2.017. Sicilian caper buds stabilized in salt were reported to possess antiradical activity of 25.8 µmol of Trolox equivalents per serving of 8.6 g using ABTS assay (Tesoriere

et al., 2007). This is equivalent to 3.0 mmol TE/kg, a value lower than those reported here for fresh caper berries. A recent study of 51 dried wild Jordanian plants, using the same assay, indicated that the antioxidant capacity ranged between 10 to 730 μmol TE/g DW (Tawaha et al., 2007). In comparison, the value reported here is within the lower range of the values reported by the Jordanians study even if values are recalculated to compensate for difference in water content.

3.2. Antioxidant constituents

3.2.1. Total free phenolics

The total phenolic content ranged from 90 to 210 mg GAE/100 g fw with an overall average of 120.0 ± 42.5 mg GAE/100 g (Table 2). Comparatively, lower total phenolic content, 37 mg GAE/100 g DW, was reported in Turkish *C. spinosa* by Aliyazicioglu et al. (2013). Salted Sicilian capers (*C. spinosa*) was reported to contain about 0.49 mg GAE/g of total phenolics (Tesoriere et al., 2007). Total phenolic content of flower buds obtained from Italy was reported to be 65 mg/g (Bonita et al., 2002). This reported value, expressed as RE, was for lyophilized defatted preparation. A recent study by a Tunisian group showed that fresh leaves and flower buds were very rich in total phenolic compounds and contained 26.2 and 36.4 mg/g FW, respectively (Tlili et al., 2010). Data from this study show that fresh fruits of *C. spinosa* were not as high in total phenolic compounds as leaves and flower buds (~25-fold less), but higher than those reported for Sicilian floral buds. Geographical, processing methods and other factors may play role in such a large variation. Several phenolic acids were found in cape buds including caffeic acid, ferulic acid, *p*-coumaric acid, and cinnamic acid (Bonita et al., 2002). Very recently, the profiling of chemical constituency, including the phenolic compounds, of buds of *C. spinosa* from India has been published (Manikandaselvi and Brindha, 2014). Fruits of *C. spinosa* can be considered as a good source of such photochemicals.

3.2.2. Total flavonoids

The overall mean ± SD of total flavonoids was 39.96 ± 10.94 mg RE/100 g FW (Table 2). Total flavonoid content represented 33% of the total phenolic compounds. Commercial capers produced in different Mediterranean countries were reported to contain 5.18 mg/g FW of flavonoid glycosides, an equivalent to 4 mg of quercetin per g (Inocencio et al., 2000). Values presented here, reported as rutin equivalent (RE), are lower than those mentioned above. Among flavonoids detected in caper buds in good proportion were quercetin and kaempferol (Inocencio et al., 2000; Sushila et al., 2010; Siracusa et al., 2011). Furthermore, leaves and buds of *C. spinosa* are rich source of lutein (Tlili et al., 2010). According to these authors, rutin constituted 37% and 26% of total phenolic compounds in leaves and flower buds, respectively. Rutin is known to possess many biological and pharmaceutical properties besides being a potent antioxidant (Siracusa et al., 2011). Two newly reported biflavonoids, isoginkgetin, and ginkgetin, were recently isolated from Chinese fruit caper by Zhou et al. (2011).

3.2.3. Total carotenoids

The overall average of total carotenoid content of caper berries was 3.91 ± 0.730 mg/100 g FW (Table 2). Very recently, the carotenoid constituents of leaves and flower buds from Tunisian *C. spinosa* have been studied by Tlili et al. (2010) who reported averages of 18.52 and 4.83 mg/100 g FW, respectively. Findings from this study were closely related to those of flower buds. Furthermore, these authors reported a high content of lutein in flower buds amounting to about 45% of total carotenoids compared to about 24% β-carotene. Lutein was not further investigated in samples of fresh caper berries studied. Carotenoids serve as antioxidant which is due to the ability to scavenge free radicals. Based on evidence presented here, fruit of *C. spinosa* can be considered as a rich source of carotenoids (Rao and Rao, 2007).

3.3. Correlation between antioxidant activities and antioxidant constituents

Table 3 shows Pearson's correlation among the methods and between the methods and the antioxidant constituents. Significant and strong correlations can be noticed. Total free phenolic content was correlated with the three assays.

3.4. Fractionation (hydrophilic and lipophilic)

In the three assays, the hydrophilic fraction of caper fruits exhibited a higher antioxidant activity (Table 1). In fact and percent-wise, the lipophilic fraction showed only 6% of that for hydrophilic fraction in the FRAP assay, and 42% and 22% in the DPPH and ABTS assays, respectively. Among the three assays employed, FRAP assay was the least suitable to detect lipophilic constituents. Both FRAP and ABTS assays were run in aqueous-based buffers (acetic and Tris–HCl, respectively), whereas DPPH assay was based on methanol. This may explain the comparatively large proportion of lipophilic fraction in the DPPH assay.

The higher antioxidant activity found in the hydrophilic fraction in the three assays is an indicative that the major contributors of the antioxidant activity in caper fruits are water soluble constituents or the phenolic compounds. Further

Table 2 Antioxidant constituents of caper berries (*C. spinosa*) collected from Bahrain. Data are mean (±SD).

Antioxidant constituent	mg/100 g fresh weight (±SD)
Total free phenolics (GAE)[a]	120.08 (42.5)
Total flavonoids (RE)[b]	39.96 (10.94)
Total carotenoids	3.91 (0.730)

[a] Expressed as gallic acid equivalent (GAE).
[b] Expressed as rutin equivalent (RE).

Table 3 Pearson's correlation coefficients.

Assay or constituent	FRAP	DPPH	ABTS
FRAP (mmol TEAC/kg fw)		0.560*	0.645**
DPPH (mmol TEAC/kg fw)			0.593**
Total phenolics (mg/100 g fw)	0.590**	0.610**	0.486*
Total flavonoids (mg/100 g fw)	0.714**	0.587**	0.652**
Total carotenoids (mg/100 g fw)	0.811**	0.561**	0.708**

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

evidence supporting this conclusion is found by summing up the antioxidant activity of both fractions in each assay. Higher values (TEAC mmol/kg FW) are obtained with FRAP (12.777) and ABTS (9.821) compared to DPPH (6.445).

3.5. Partitioning of antioxidant activity

When the edible content of the *C. spinosa* fruit was separated into flesh and seed, methanolic seed extract prepared from a pooled sample exhibited remarkably higher antioxidant activity than flesh extract prepared from the same pooled sample in the three assays employed (Table 1). The average antioxidant values possessed by seed extract, measured by FRAP, DPPH, and ABTS assays and expressed as TEAC mmol/kg FW, were 17.76 ± 0.0377, 6.628 ± 0.261, and 13.214 ± 0.321, respectively. For flesh, the values were 7.92 ± 0.151, 2.215 ± 0.106 and 6.392 ± 0.478, and for whole fruit the values were 14.390 ± 0.085, 5.537 ± 0.470, and 9.424 ± 0.0.153, respectively. Compared to the antioxidant values of seed measured by FRAP, DPPH, and ABTS assays, flesh possessed only 45%, 31%, and 48%, respectively. Furthermore, the antioxidant activity of the whole content (seed and flesh combined) was lower than the seed and amounted to only 81%, 86%, and 71%, respectively. Total free phenolics and total flavonoid contents were both higher in seed extract than the flesh alone or the combined sample, which indicate that higher antioxidant activity found in seeds may be attributed to the presence of phenolic compounds.

3.6. Seasonal variation of FRAP value

Fig. 1 shows the FRAP value of the ripped caper fruit during six successive seasons covering the period 2002–2012. The FRAP value varied considerably and ranged between 5 and 12 mmol TEAC/kg FW. The highest FRAP values were reported during seasons 2008 and 2010. These two seasons were characterized by comparatively high rainfall, 374 and 126 mm. Lower FRAP values were recorded during seasons of low rainfall; seasons 2002 (56 mm), 2004 (79 mm), and 2012 (13 mm). The average annual rainfall in Bahrain is 78 mm. It should be noted, however, that the relationship between the antioxidant activity, expressed as FRAP value, and the precipitation rate was not systematically investigated.

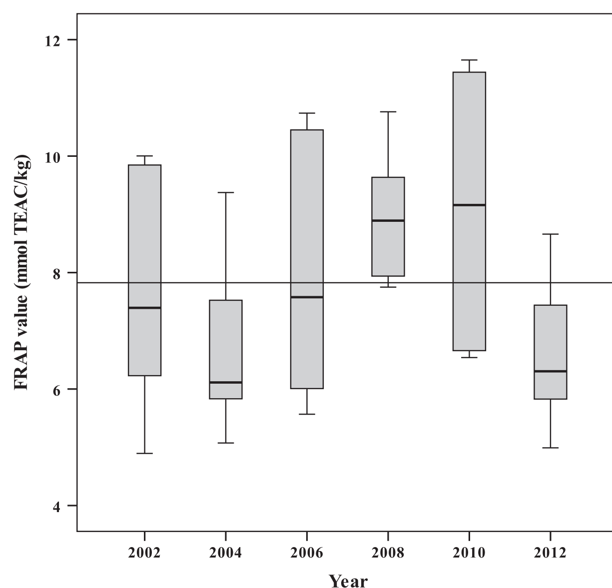

Figure 1 A whistle-box plot of FRAP value of the edible content of the *C. spinosa* during the period 2001–2012. For each given year, fruits were collected from various locations during the fruiting period (September to November) and analyzed as described in Experimental Section. The reported FRAP values represent the average of 5–6 composite samples. The horizontal line represents the overall average during the ten-year period.

4. Conclusions

The current economic importance of *C. spinosa*, supported by the current upsurge in scientific interest for exploring natural food sources rich in bioactive compounds, has stimulated intense research into the photochemical aspects and potential usage of caper berries. Results from this study indicate that the edible portion of fresh caper berries possesses a high antioxidant activity which is comparable to many other wild berries. Caper berry is a good source of antioxidant compounds including free phenolics, flavonoids, and carotenoids. Partitioning the activity revealed that hydrophilic constituents contribute more toward the antioxidant property of the fresh fruit. Fractionation experiment showed that greater antioxidant activity was located in fruit seeds.

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