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Research Article

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Phytochemical Investigation and *in vitro* Antioxidant Activities of *Cleome amblyocarpa* and *Cleome ramosissima* growing in Saudi Arabia

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ABSTRACT

In this study, the phytochemical composition, total phenolic contents (TPC), total flavonoids contents (TFC) and antioxidant activity of crude fractions from aerial parts of Cleome amblyocarpa and C. ramosissima growing in the Kingdom of Saudi Arabia were investigated. The antioxidant activity of all extracts tested was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, 2, 2'–Azino–bis (3-ethylbenzoline -6- sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) free radical, Metal chelating activity (MCA), and superoxide radical anion ($O^{\bullet-}_2$) scavenging assays. Considerable amounts of TPC and TFC were measured in the extract fractions from the two Cleome species. The butanol extract from each species yielded the greatest antioxidant activity. In addition Phytochemical screening extracts of the above plants revealed the presence of tannins, alkaloids, terpenes, saponins, flavonoids, anthraquinone and glycosides.

Keywords: *Cleome amblyocarpa*; *Cleome ramosissima***;** Antioxidant activity**;** Alkaloids**;** Phenolic content**;** Flavonoid content, DPPH; $ABTS^{\bullet+}$

INTRODUCTION

Medicinal plants are widely used as alternative therapeutic tools for the prevention or treatment of many diseases. Accumulation of free radicals can cause pathological conditions such as ischemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementia. Natural antioxidants have become the target of a great number of research studies in seeking for the sources of potentially safe, effective, and cheap antioxidants [1]. Herbal drugs containing free radical scavengers are known for their therapeutic activity [2]. The use of plant products as antioxidants in processed food is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants [3-6]. Recent studies underscored that the antioxidant effect of plant products is mainly attributed to phenolic compounds such as flavonoids, phenolic acids and tannins [7,8]. Antioxidants are significant regarding reducing oxidative stress which could affect and damage biological molecules [9]. Oxidative stress is the disproportion between oxidants and antioxidants in favor of oxidants potentially leading to damage. Reactive oxygen species (ROS) are a class of compounds that are formed from oxygen metabolism. These highly reactive molecules such as, hydroxyl radical ($^{\bullet}OH$), peroxide ($ROO^{\bullet}$) and superoxide radicals (O^{2-}), can cause severe damage to cells and tissues during various diseases which are linked to heart disease, carcinogenesis and many other health issues. Synthetic antioxidants such as butylated hydroxyl anisole (BHA), propyl gallate (PG), butylated hydroxyl toluene (BHT) which have been used to prevent oxidation have been found to cause internal and external bleeding in rats and guinea pigs at high dose [10-12]. *Cleome* L. (Cleomaceae), a genus of ca. 200 pantropical and temperate species [13], has a long history of ethnomedicinal usage [14]. *Cleome* species are

generally used in folk medicine as stomachics, rubefacients and in the treatment of scabies, rheumatic fever and inflammation [15-18]. *Cleome viscosa* L., one of the most common medicinal herbs throughout India, is used as a remedy to treat various ailments [19]. Phytochemical screening studies underscored *Cleome* enrichment with a diverse array of beneficial secondary products including terpenoids, flavonoids, phenolics, and alkaloids, supporting use of the genus for culinary and therapeutical purposes [20-22]. However, information about allelochemicals in *Cleome* species is limited. In addition, little has been reported on flavonoids from *Cleome* species with only two species being studied so far namely *C. viscosa* and *C. droserifolia* [23-25].

Five *Cleome* species are known to grow wild in Saudi Arabia, including *C. amblyocarpa*, *C. arabica* L., *C. ramosissima*, *C. chrysantha* and *C. viscosa* L. [26]. This species are generally used in folk medicine in Saudi Arabia as antimicrobial, carminative, anthelmintic, antiseptic, sudorific, irritant, acrylic, rubefacient and vesicant [27-29]. It is known that the *C. amblyocarpa* cause nervous disorders in animals [18], which is an important medicinal plant in Tunisia where it is widely used against colic and diabetes [18]. It was investigated previously [30], only the presence of two dammarane-type triterpenes [31] with stigma-4-en-3-one, lupeol and taraxasterol as well as a cembrane derivative [32]. To the best of our knowledge, investigations on *Cleome* species from Saudi Arabia origin for phytochemical content and antioxidant activity have not been carried out. Therefore, we aimed in this study to phytochemical screening and examine the antioxidant activity by DPPH, ABTS, Metal chelating activity and hydroxyl assays from two *Cleome* species (*C. amblyocarpa* and *C. ramosissima*) growing wild in Saudi Arabia.

MATERIALS AND METHODS

Plant Materials

Fresh Aerial parts of *C. amblyocarpa* and *C. ramosissima* were collected from Widi Nissah, Howtha Banu Tamim, Huraimla and Salbouk, Saudi Arabia, during the flowering period April, 2015. The plants were taxonomically identified and authenticated by the Botanical Survey Division of Dr Jacob Thomasfrom the Herbarium Division, College of Science, King Saud University, Riyadh, KSA. The fresh aerial parts of plants materials were dried at room temperature in a shady place for a month.

Instrumentation

The absorbance of the resulting mixture was measured by using Biochrom WPA Wavelight II UV- visible spectrophotometer.

Materials and Chemicals

DPPH "2,2 Diphenyl -1 - picrylhydrazyl" (Sigma- Aldrich), ABTS "2, 2' – Azino – bis (3- ethylbenzoline -6-sulfonic acid), diammonium salt" (Sigma- Aldrich), Folin&Ciocaltea's phenol reagent (Sigma- Aldrich), Ferrozin "3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p′-disulfonic, acid monosodium salt hydrate" (Sigma- Aldrich). $FeCl_2$ (VWR), $K_2S_2O_8$ (fluka), NaOH (Sigma- Aldrich), $AlCl_3$, Na_2CO_3 (Sigma- Aldrich), $NaNO_2$ (Sigma- Aldrich) H_2O_2, $FeSO_4$, Salicylic acid (Sigma- Aldrich)

Extraction of Antioxidants

The air dried powdered plant samples were extracted in soxhlet extractor successively with petroleum ether to remove the fatty acids followed by methanol. Each time before extracting with the next solvent, the material was dried. The extracts were concentrated by rotary vacuum evaporator and then dried. This residue was partitioned between $CHCl_3$ and H_2O (1:1) solvent system. After the separation of $CHCl_3$ and H_2O phases, the dried $CHCl_3$ fraction was partitioned between 10% aqueous methanol and hexane. The polar organic compounds were extracted from water by n-butanol. The extracts thus obtained were used directly for the estimation of total phenolic and for the assessment of antioxidant potential through various chemical assays.

Phytochemical Screening

The crude extracts obtained from *C. amblyocarpa* and *C. ramosissima* were tested for the presence of flavonoids, alkaloids, terpenes, saponins, glycosides, anthraquinone and tannins according to the procedures described in the literatures [33-35]. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

Determination of the Total Flavonoid

The total flavonoid contents of two spices of cleome in different extracts were determined using the aluminium chloride assay through colorimetry [36]. Aliquits of (1mL) extracts of (1mg/mL) concentration were taken in different volumetric flask(10mL) then 4mL of distilled water was added followed by the addition of 0.3 mL of sodium nitrite (5% $NaNO_2$, w/v) and allowed to stand for 5 min. Later 0.3 mL of aluminium chloride (10% $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 mL of sodium hydroxide (NaOH, 1 M) and volume was made up to the 10 mL with distilled water. After 15 min absorbance was measured at 510 nm. Methanol was used as blank.

Determination of Total Phenolic Content

The total phenolic content will be analyzed by the Folin–Ciocalteu method as described by Singleton et al. [37] and for this purpose, 0.5 mL of extract was treated with 2.5 mL of Folin–Ciocalteu reagent (2N) (diluted ten folds) and 2 mL of Na_2CO_3 (75 g/L). The mixture was allowed to stand at room temperature for 15 min and the absorbance was recorded with a spectrophotometer (UV-Vis) at 765 nm wavelength with respect to the blank solution methanol. Gallic acid was used as a standard for calibration curve, by measuring its absorbance at different concentrations (0.4-2 mg/mL). All measurements were performed in triplicates. Total phenolic content expressed as mg/g gallic acid equivalent.

DPPH• Free Radical Scavenging Activity

The total radical scavenging capacity of the extracts of *C. amblyocarpa* and *C. ramosissima* were determined and compared to that of ascorbic acid and α- tocopherol which were used as standards according to the literatures [38,39]. A 0.1mM solution of DPPH• was prepared in methanol and 2 mL of this solution was added to 1 mL of extract solution in methanol at different concentrations (0.005 -1.0) mg/mL. These solutions were incubated in the dark for 30 min. Then the absorbance was measured at 517 nm against blank samples lacking scavenger, all experiments were repeated three times. A standard curve was prepared using different concentrations of DPPH•. The ability to scavenge the DPPH• radical was calculated using the following equation:

DPPH• scavenging effect (%) =Ac-As/Ac *100%

Where, AC is the absorbance of the blank and AS is the absorbance in the presence of extract. [DPPH•] decreases significantly upon the exposure to radical scavengers. A parameter introduced for the interpretation of the results from the DPPH method was the IC_{50} value. This is defined as the concentration of the substrate that causes 50 % loss of the DPPH activity.

ABTS Radical Scavenging Assay

The total antioxidant activity by radical cation ($ABTS^{\cdot+}$) decolonization assay was determined according to the literature [39,40]. The $ABTS^{\cdot+}$ cation radical solution was prepared by reacting similar quantities of 7mM of ABTS and 2.4 mMof potassium persulphate ($K_2S_2O_8$) solutions for 16 hours at (2-3ºC) in the dark. Before using this solution, it was diluted with distilled water to obtain an absorbance of 0.75 ± 0.02 at 734 nm. The reaction mixtures composed of 3mL of $ABTS^{\cdot+}$ solution and 1mL of extracts at different concentrations (0.005 - 1.0) mg/mL. The absorbance was measured at 734 nm by using UV-VIS spectrophotometer. The blank was run in each assay and all measurements were taken after at least 5 min. The ABTS scavenging capacity of extract was compared with that of ascorbic acid and α tocopherol and percentage inhibition was calculated as: = Ac-As/Ac *100%

Where, AC is the absorbance of the blank and AS is the absorbance in the presence of extract.

Metal Chelating Activity

The chelating of ferrous ion by extracts was estimated by the method mentioned in the literature [41] with little modifications. Fe^{2+}-chelating ability of extracts was monitored by the absorbance of the ferrous iron–ferrozine complex at 562 nm. {3 mL of extract solution in methanol at different concentrations (0.005 - 1.0) mg/mL} was added to the solution of 2mM $FeCl_2$ (250 µL). The reaction was initiated by the addition of 5mM ferrozine (0.2 mL). Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated by using the equation given below:

Metal-chelating activity (%) = (Ac - As)/ Ac × 100

Where, AC is the absorbance of the control and AS is the absorbance in the presence of extracts or standards. The control contains only $FeCl_2$ and ferrozine.

Hydroxyl Radical Assay

Fenton reaction is the typical model system to produce .OH *in vitro*. Salicylic acid was used to measure the hydroxyl radical formation according to previously reported method [42]. 1 mL of extract solution in methanol at different concentrations (0.005 - 1.0) mg/mL was added to a solution of 0.5mL $FeSO_4$ (6 mM) then 0.5mL H_2O_2 (6 mM). The tube was shaken well and left standing for 10 min. Then 1mL salicylic acid (6mM) was added and incubated for 30 min at room temperature. VitC was used as positive control. The absorbance was measured at 510 nm. The hydroxyl radical scavenging ability was calculated: = (Ac - As)/ Ac × 100

Where AC is the absorbance of the control and AS is the absorbance in the presence of extracts or standards.

Statistical Analysis

All the results in the current study were calculated using (Microsoft Excel 2010) and recorded in triplicate. Each value represents the mean ± S.D of three samples and all bars in the figures represent S.D. The IC_{50} was determined from the sigmoidal curve obtained by plotting the scavenging ability relative to the control versus logarithmic concentration of test extracts using non-linear regression analysis of GraphPad Prism 6 (GraphPad Software, San Diego, California, USA). Each concentration was tested trice in 3 independent experiments.

RESULTS AND DISCUSSION

The phytochemical screening of the crude extracts from *C. amblyocarpa* showed that alkaloids are present in all, whereas the flavonoids, saponins and tannins are present in the butanol, aq.MeOH, and water crude extracts. Terpenes were found in the butanol and aq. MeOH extracts of *C. amblyocarpa*. However, all extracts of *C. amblyocarpa* did not show any colour change for anthraquinone test. By comparison, aq.MeOH and butanol crude extracts from *C. ramosissima* were found to contain alkaloids, flavonoids, glycosides, saponins and tannins. However, terpenes were detected in the butanol crude extract from *C. ramosissima*. The water crude extract included flavonoids and Glycosides. Unlike the hexane crude fraction from *C. amblyocarpa*, the hexane crude extract from *C. ramosissima* showed negative test for all groups (Table 1).

Table 1: Major phytochemical groups detected in crude extract fractions of *C. amblyocarpa* and *C. ramosissima*

Groups	Aq.MeOH		Butanol		Water		n-Hexane	
	C. amblyocarpa	*C. ramosissima*	*C. amblyocarpa*	*C. ramosissima*	*C. amblyocarpa*	*C. ramosissima*	*C. amblyocarpa*	*C. ramosissima*
Alkaloids	+	+	+	+	+	-	+	-
Tannins	+	+	+	+	+	-	-	-
Glycosides	+	+	-	+	+	+	-	-
Flavonids	+	+	+	+	+	+	-	-
Sponins	+	+	+	+	+	-	-	-
Terpenes	+	-	+	+	-	-	-	-
Anthraquinone	-	-	-	-	-	-	-	-

The amount of total phenolics varied in the different extracts and ranged from 30.33 to 102.75 mg/g Gallic acid of *C. amblyocarpa* and from 20.17 to 139.50 mg mg/g Gallic acid of *C. ramosissima* (Table 2). Among the four crude extracted of *C. amblyocarpa*, the butanol extract showed the highest amount of phenolic compounds. Polar fractions had more phenolics than had non-polar fractions. However, the aq.MeOH extract from *C. ramosissima* showed the highest amount of phenolic compounds. Similar results were noted when the lowest amount of phenolics was recorded in non-polar extracts from aerial parts of *C. ramosissima*. In this study, the butanol extract of *C. amblyocarpa* had a higher flavonoids content compared to the other extracts, whereas, the aq.MeOH extract of *C. ramosissima* had a higher flavonoids content compared to the other extracts. The hexane had the lowest capacity to extract flavonoids from the two plants (Table 2).

Table 2: Total phenolic content and total flavonoids of all extracts of *C. amblyocarpa* and *C. ramosissima*

Extracts	Total Phenol (mg/g Gallic acid)		Total flavonoids (mg/g Quercetin acid)	
	C. amblyocarpa	*C. ramosissima*	*C. amblyocarpa*	*C. ramosissima*
Butanol	102.75 ± 0.75	28.50 ± 0.41	194.56 ± 1.57	229.00 ± 4.50
Aqueous methanol	55.67 ± 1.89	139.50 ± 0.62	122.34 ± 4.71	745.66 ± 6.56
Water	32.83 ± 0.85	113.45 ± 1.71	5.67 ± 0.41	105.67 ± 3.34
Hexane	30.33 ± 0.85	20.17 ± 0.24	ND	27.89 ± 1.57

[a]Values expressed are means ± S.D. of three parallel measurements

There are many methods used to evaluate the free radical scavenging activity of compounds [43]. The antioxidant activities of plant extracts vary with the assay methods because of the complex nature of phytochemicals present in them and the solvent used for extraction [43]. The methods selected were those most commonly used for the determination of antioxidant activities of plant extracts. In the present study, DPPH, ABTS radical scavenging activity, hydrogen peroxide scavenging and Metal chelating activity were evaluated in *C. amblyocarpa* and *C. ramosissima* extracts (butanol, aqueous methanol, water and hexane). The free radical scavenging activities of butanol, aqueous methanol, water and hexane extracts of *C. amblyocarpa* and *C. ramosissima* were tested by the DPPH method (Table 3). The activity was concentration dependent shown Figure 1. The IC_{50} values of DPPH free radical scavenging activity are shown in Table 3. The butanol extract of *C. amblyocarpa* and *C. ramosissima* showed a smaller value of IC_{50} ($0.11 \pm 6.60*10^{-3}$and 0.12 ± 0.006 [mg/mL], respectively), which was higher that of standard α-Tocopherol and ascorbic acid ($IC_{50} = 0.019 \pm 2.36*10^{-3}$ and $7.22*10^{-5} \pm 2.30*10^{-6}$ mg/mL). These results indicate that the butanol extract had higher antioxidant activity than the other extracts. The aqueous methanol extract of *C. amblyocarpa* and *C. ramosissima* showed an IC_{50} value of $0.13 \pm 8.17*10^{-3}$ and $0.34 \pm 4.70*10^{-3}$ (mg/mL), respectively, while the water extract showed minimum scavenging activity with a high IC_{50} value (0.30 ± 0.03 and 0.43 ± 0.06 µg/mL) (Table 3). The ABTS radical cation scavenging activities of *C. amblyocarpa* and *C. ramosissima* are shown in Table 3. The activity was concentration dependent (Figure 2), and the maximum scavenging activity was found in the butanol extract of *C. amblyocarpa* ($IC_{50} = 9.50*10^{-2} \pm 0.02$ mg/mL) and *C. ramosissima* ($IC_{50} = 7.53*10^{-2} \pm 5.44*10^{-3}$ mg/mL), which was near that of standards α-Tocopherol ($IC_{50} = 0.054 \pm 2.86*10^{-3}$ mg/mL) and ascorbic acid ($IC_{50} = 0.010 \pm 0.00$ mg/mL), followed by aqueous methanol extract (Table 3). The antioxidant activity of different extracts as equivalent to DPPH was in the order of butanol > aqueous methanol > water extract> hexane extract for to *C. amblyocarpa* and *C. ramosissima*. Therefore, the antioxidant activity results for all crude extracts from *C. amblyocarpa* were higher than that from *C. ramosissima* (Table 3).

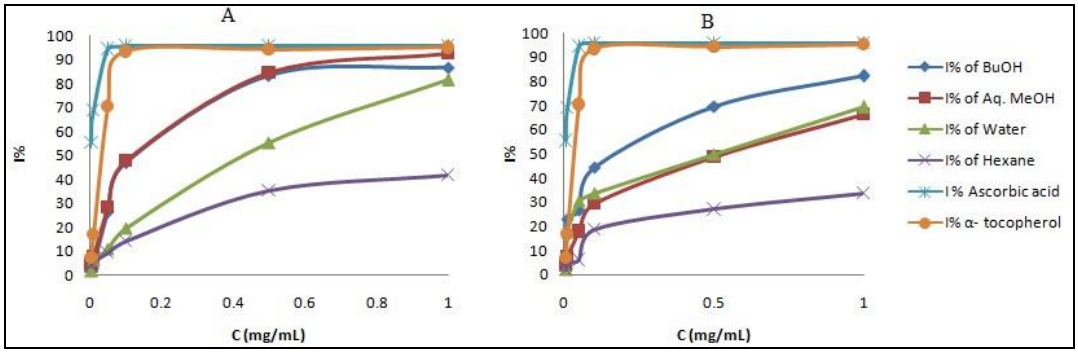

Figure 1: Antioxidant activity of the extract fractions of *C. amblyocarpa* (A), *C. ramosissima* (B) and positive controls (ascorbic acid and α-Tocopherol) on DPPH· assay, I%: radical scavenging activity. Values expressed are means of three parallel measurements

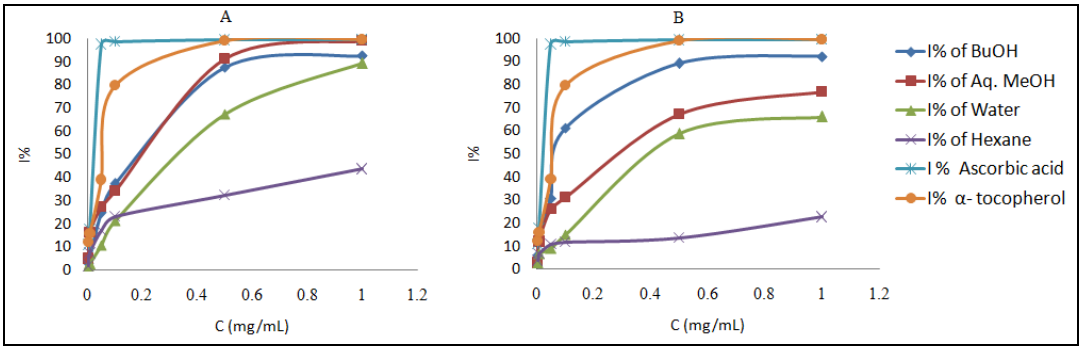

Figure 2: Antioxidant activity of the of the extract fractions of *C. amblyocarpa* (A), *C. ramosissima* (B) and positive controls (ascorbic acid and α-Tocopherol) on $ABTS^{+\cdot}$ assay. I%: radical scavenging activity. Values expressed are means of three parallel measurements

Table 3: Antioxidant activities of extracts from *C. amblyocarpa* and *C. ramosissima* using DPPH and ABTS methods, α-Tocopherol and Ascorbic acid were used as positive controls

Extracts	DPPH IC_{50} (mg/mL)		ABTS IC_{50} (mg/mL)	
	C. amblyocarpa	*C. ramosissima*	*C. amblyocarpa*	*C. ramosissima*
Butanol	$0.11 \pm 6.60*10^{-3}$	0.12 ± 0.006	$9.50*10^{-2} \pm 0.02$	$9.53*10^{-2} \pm 5.44*10^{-3}$
Aqueous methanol	$0.13 \pm 8.17*10^{-3}$	$0.34 \pm 4.70*10^{-3}$	$9.70*10^{-2} \pm 8.90*10^{-3}$	$0.19 \pm 2.16*10^{-3}$
Water	0.30 ± 0.03	0.43 ± 0.06	$0.21 \pm 9.9*10^{-3}$	0.47 ± 0.03
Hexane	nd	nd	nd	nd
α-Tocopherol	$0.019 \pm 2.36*10^{-3}$		$0.054 \pm 2.86*10^{-3}$	
Ascorbic acid	$7.22*10^{-5} \pm 2.30*10^{-6}$		0.010 ± 0.00	

%I: Radical scavenging activity; [a]Values expressed are means ± S.D. of three parallel measurements

Some redox-active transition metals such as iron (Fe) are essential elements required for the growth and survival of mammals, whereas they also play the catalytic role in the free radical formation and cause various oxidative damages [41]. The above-mentioned Fenton reaction is just involved by ferrous iron (Fe^{2+})-dependent decomposition of hydrogen peroxide to generate hydroxyl radicals. Accordingly, the Fe^{2+} chelating activity is also an important attribute of the antioxidants. As shown in Figure 3, the chelating effects of all extracts were ascended with the increase of concentrations. Based on the IC_{50} values, the ranking order of the activity was butanol > water extract > aqueous methanol > hexane extract for to *C. amblyocarpa* and butanol > aqueous methanol > water extract> hexane extract for to *C. ramosissima*, which corresponded to their flavonoids and glycosides contents (Table 1).

Hydroxyl radicals are highly reactive towards proteins, lipids and DNA, and severely harmful for cell survival when overproduced [42]. Removal of the radicals is thus important for the living systems to maintain the redox homeostasis. As shown in Figure 4, the scavenging activity of the extract of *C. amblyocarpa* and *C. ramosissima* increased with increase in the concentration of the extract. According to the IC_{50} values, the order of the scavenging activity was aqueous methanol > butanol > water extract > hexane for the *C. amblyocarpa* and butanol > aqueous methanol > water extract > hexane extract for to *C. ramosissima* (Table 3).

CONCLUSION

In the present study, the phytochemical screening, total phenolic contents (TPC), total flavonoids contents (TFC) and antioxidant activity of crude fractions from aerial parts of *C. amblyocarpa* and *C. ramosissima* growing in the Kingdom of Saudi Arabia were investigated. There was a direct relation between antioxidant activity and the content of active compounds, phenol and flavonoid in some extracts in this study. Some extracts with high phenol and flavonoid contents showed good antioxidant activity. For example, the butanol extract of *C. amblyocarpa* that contained highest phenol and flavonoid contents showed the best chelating activity. Also, aqueous extract of *C. ramosissima* showed good activity. All extracts showed a variety of activity and phytochemical compounds in this study.

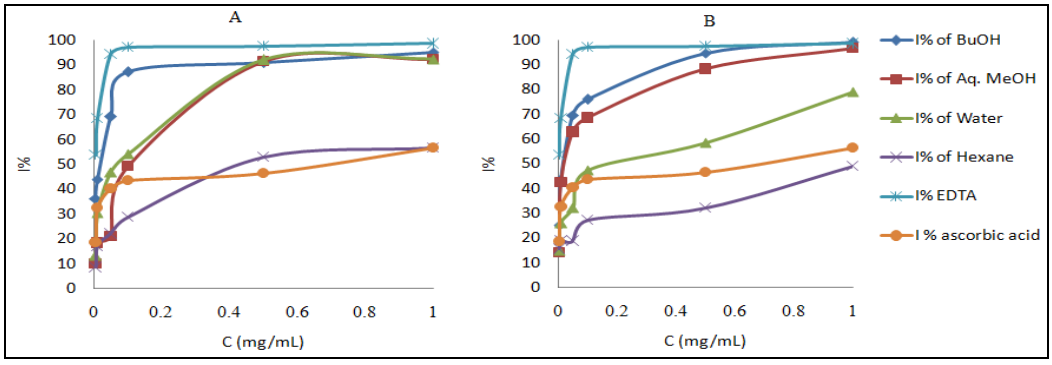

Figure 3: Metal chelating effects on ferrous ions of the extracts from *C. amblyocarpa* (A), *C. ramosissima* (B) and positive controls (EDTA and ascorbic acid) I%: Radical scavenging activity. Values expressed are means of three parallel measurements

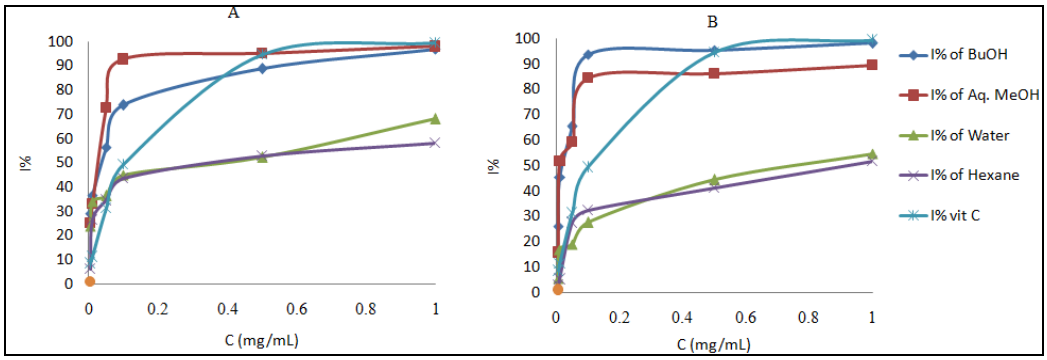

Figure 4: Hydroxyl radical scavenging activity of the crude extracts of *C. amblyocarpa* (A), *C. ramosissima* (B) and positive control (vit C), I%: Radical scavenging activity. Values expressed are means of three parallel measurements

Table 4: Metal chelating effect and Hydroxyl radical assay of all extracts of *C. amblyocarpa* and *C. ramosissima*. EDTA and ascorbic acid were used as positive controls

Extracts	Metal chelating effect IC50 (mg/ml)		Hydroxyl radical assay IC50 (mg/ml)	
	C. amblyocarpa	*C. ramosissima*	*C. amblyocarpa*	*C. ramosissima*
Butanol	$1.10*10^{-2} \pm 9.40*10^{-3}$	$1.53*10^{-2} \pm 4.70*10^{-4}$	$2.48*10^{-2} \pm 2.20*10^{-3}$	$1.80*10^{-2} \pm 1.60*10^{-3}$
Aqueous methanol	$8.10*10^{-2} \pm 1.60*10^{-3}$	$0.02 \pm 1.24*10^{-3}$	$1.86*10^{-2} \pm 2.30*10^{-3}$	$2.90*10^{-2} \pm 1.69*10^{-3}$
Water	$5.10*10^{-2} \pm 2.89*10^{-3}$	$5.10*10^{-2} \pm 2.89*10^{-3}$	0.18 ± 0.02	$0.13 \pm 7.50*10^{-3}$
Hexane	0.56 ± 0.04	0.56 ± 0.04	0.21 ± 0.01	0.88 ± 0.02
EDTA	$5.58*10^{-4} \pm 3.31*10^{-5}$		-	
Ascorbic acid	0.42 ± 0.08		$0.11 \pm 2.13*10^{-3}$	

%I: Radical scavenging activity; [a]Values expressed are means ± S.D. of three parallel measurements

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