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Polyphenolic content and antioxidant activity of some wild Saudi Arabian asteraceae plants

Abdelaaty A. Shahat[1,2*], Abeer Y. Ibrahim[3], Mansour S. Elsaid[1]

[1]*Pharmacognosy Department, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia*
[2]*Phytochemistry Department, National Research Centre, El-Bohous Street, 12622, Dokki, Egypt*
[3]*Medicinal and Aromatic Plants Research Dept., National Research Centre, El-Bohous Street, 12622, Dokki, Egypt*

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ABSTRACT

Objective: To study the antioxidant properties of crude extract of different Asteraceae plants. **Methods:** The antioxidant properties of six extracts were evaluated using different antioxidant tests, including free radical scavenging, reducing power, metal chelation, superoxide anion radical scavenging, total antioxidant capacity and inhibition of lipid peroxidation activities. **Results:** *Picris cyanocarpa* (*P. cyanocarpa*) and *Anthemis deserti* (*A. deserti*) had powerful antioxidant properties as radical scavenger, reducing agent and superoxide anion radical scavenger while *Achillia fragrantissima* (*A. fragrantissima*) and *Artemissia monosperma* (*A. monosperma*) were the most efficient as ion chelator (100% at 100, 200 and 400 μg/mL) *A. fragrantissima* and *Rhantarium appoposum* (*R. appoposum*) showed 100% inhibition on peroxidation of linoleic acid emulsion at 200 and 400 μg/mL, while butylatedhydroxy toluene and ascorbic acid showed 100 and 95% inhibition percentage at 400 μg/mL, respectively. Those various antioxidant activities were compared to standard antioxidants such as butylated hydroxyl toluene and ascorbic acid. **Conclusions:** In most tests *P. cyanocarpa* and *A. deserti* had powerful antioxidant properties as radical scavenger, reducing agent and superoxide anion radical scavenger.

1. Introduction

Medicinal plants, herbs and fruits with a high content of bioactive compounds and related antioxidant capacity are inversely associated with morbidity and mortality from atherosclerosis in general, and coronary atherosclerosis in particular[1-5]. It has been shown that the best health and nutrition results can be achieved from theconsumption of plants with high antioxidant activities[6,7]. Dietary bioactive compounds and microelements from different functional foods, herbs and nutraceuticals (ginseng, ginkgo, nuts, grains, tomato, soy phytoestrogens, curcumin, melatonin, polyphenols, antioxidant vitamins, carnitine, carnosine and ubiquinone) can ameliorate or even prevent diseases[8,9].

Recently, there has been considerable interest in finding naturally occurring antioxidant to replace synthetic antioxidants in foods. The addition of synthetic antioxidant in food and beverage may impose health risks resulting in strict regulations over their use in foods[10].

In terms of biodiversity, the flora of Saudi Arabia represents one of the richest areas in the Arabian Peninsula and comprises a very important genetic resource of crop and medicinal plants. 8 out of 27 extracts of selected Saudi Arabian medicinal plants, 5 of which belong to the Asteraceae family, have the ability to induce the cytoprotective marker enzyme NQO1[11]. In the present study we decided to evaluate the antioxidant activity of six Asteraceae medicinal plants collected from localities of Saudi Arabia. The antioxidant activity of sequential extracts was tested using many reactions.

*Corresponding author: Abdelaaty A. Shahat, Pharmacognosy Department, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia.
Tel: +966 537507320
Fax: +966 1 4676220
E-mail: aashahat@hotmail.com
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2. Materials and methods

2.1. Plant material

All plants (Table 1) were collected from the Tanhat protected area except Artemisia monosperma which was collected from Aldahna, Saudi Arabia in April 2012. The plants were identified by the Plants Taxonomist at the Herbarium Unit. The voucher specimens have been deposited at the Herbarium of the Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

2.2. Sample preparation

The plants were collected and dried under shade. The dried samples were powdered and used for solvent extraction. For extract preparation, 100 g of dried sample was extracted twice with 300 mL of 80% methanol. The extracts were filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator under reduced pressure at 40 ℃. The dry extract obtained with each solvent was weighed. The percentage yield was expressed in terms of air dried weight of plant materials

2.3. Antioxidant activities

2.3.1. Chemicals

Ammonium thiocyanate, 1,3-diethyl thiobarbituric acid (DETBA) and Linoleic acid were purchased from E. Merck. Ferrous chloride, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), nicotinamide adenine dinucleotide (NADH), butylatedhydroxytoluene (BHT), ABTS (2,2-azino-bis(3-ethylbenzthiazolin-6-sulphonic acid) diammonium salt), peroxidase, potassium hexacyanoferrate, and trichloroacetic acid (TCA) were purchased from Sigma (Sigma-Aldrich).

2.3.2. Total polyphenols content

The Folin Ciocalteu method was used and the measurement was performed at 765 nm with gallic acid as the standard[12]. The results were expressed as grams of gallic acid equivalent (GAE) per 100 g of dry weight (DW).

2.3.3. DPPH radical scavenging activity determination

The effects of crude extracts and positive controls (Ascorbic acid and BHT) on DPPH radicals were estimated based on the method of Yamaguchi[13]. Aliquots (20 μL) of crude extracts at various concentrations (25, 50, 100, 200 and 400 μg/mL) were each mixed with 100 mM Tris-HCl buffer (80 μL, pH 7.4) and then with 100 μL of DPPH in ethanol to a final concentration of 250 μM. The mixture was shaken vigorously and left to stand at room temperature for 20 min in the dark. The absorbance of the reaction solution was measured spectrophotometrically at 517 nm. The percentage of DPPH decolorization of the samples was calculated according to the equation:

% decolorization = [1- (ABS sample /ABS control)] ×100.

IC_{50} value was the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis. A lower IC_{50} value indicated a greater antioxidant activity.

2.3.4. Total reduction capability

Total reduction capability of plant extracts was estimated by using the method of Oyaizu[21]. Plant extracts (25-400 μg/mL) in 1 mL of methanol (HPLC grade) was mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50 ℃ for 20 min by adding a 2.5 mL of 10% trichloroacetic acid. Then the mixture was centrifuged for 10 min at 1 000 × *g*. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm by a spectrophotometer (SchimadzuUV/Vis-240IPC). Higher absorbance of the reaction mixture indicated greater reducing power.

2.3.5. Ferrous ion chelating activity

The ferrous ions chelating by the different plant extracts concentrations and standards were estimated by the method

Table 1
Some Asteraceae plants used in the present study [11]. *80% methanol extracts from the aerial part.

	(Plant species) (Voucher specimen)	Traditional use	Sample used (yield in %)*
1	*Pulicaria crispa* (15934)	Treat inflammation and an insect repellent and is also used as an herbal tea[14].	6.7%
2	*Rhantarium epapposum* (15935)	Skin infections and gastrointestinal disturbances and as an insecticide[15].	3.1%
3	*Picris cyanocarpa* (15939)	Treatment of indigestion, against intestinal nematodes and other parasites[16].	19.5%
4	*Anthemis deserti* (15940)	Herbal medicines, insecticides, and dyes, food additives, as well as an important source in aromatic and cosmetic industries[17].	10.8%
5	*Achillia fragrantissima* (15952)	Respiratory diseases and gastrointestinal disturbances[18].	6.2%
6	*Artemisia monosperma* (15960)	Antispasmodic, anthelmintic and anti-hypertensive[19].	16.4%
7	*Achillea beibersteni* (15470)	Spasmolytic, choleretic, treatment of wounds and anti-inflammatory activities, make it as an important medicinal plant[20].	10.4%

of Dinis[22]. Briefly, the samples (25-400 μg/mL) were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm using a UV-Visible Spectrophotometer (Schimadzu UV/Vis-240IPC). All tests and analyses were run in triplicate andaveraged. The percentage of inhibition of ferrozine. Fe^{2+} complex formation was found using the following formula:

%inhibition = $[(A_0-A_1)/A_0] \times 100$.

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the standard sample or seven plant extract concentrations. The control contained $FeCl_2$ and ferrozine complex formation molecules.

2.3.6. Superoxide anion radicals scavenging activity

Measurement of superoxide anion radicals scavenging activity of different plant extract concentrations was based on the method describedby Liu[23]. Superoxide radicals are generatedin PMS-NADH systems by oxidation of NADH and assayed by the reduction of NBT. In these experiments, the superoxide radicals were generated in 3 mL of Tris-HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT(50 μM), 1 mL NADH (78 μM) and different plant extracts (25-400 μg/mL). The reaction was started by adding 1 mL of PMS solution (10 μM) to the mixture. The reaction mixture was incubated at 25 ℃ for 5 min, the absorbance was read at 560 nm using a spectrophotometer (Schimadzu UV/Vis-240IPC) against blank samples using L-ascorbic acid and BHT as standards. Decreasing absorbance of the reaction mixture indicated increasing superoxide anion scavenging activity.

The percentage inhibition of superoxide anion generation was calculated using the following formula:

% inhibition = $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of the control (L-ascorbic acid), and A_1 was the absorbance in the presence of plant extracts or standards.

2.3.7. Total antioxidant activity

Total antioxidant activity was measured according to the method described by Miller and Rice-Evans[24] and Arnao, Cano, and Acosta[25]. Exactly 0.2 mL of peroxidase (4.4 units/mL), 0.2 mL of H_2O_2 (50 μM), 0.2 mL of ABTS (2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid, diammonium salt, 100 μM) and 1 mL distilled water were mixed, and were kept in the dark for 1 h to form a bluish green complex. After adding of 1 mL plant extracts of different concentrations, extracts were tested in triplicates. The absorbance at 734 nm was measured to represent the total antioxidant activity and then was calculated as follows:

Total antioxidant activity (%) = $[1-(A_{sample}/A_{control})] \times 100$

2.3.8. Inhibition of lipid peroxidation

The potential of plant extracts at different doses was determined according to the method of Gulcin[26] to inhibit peroxidation of linoleic acid. L-ascorbic acid and BHT were used as the reference compounds. A pre-emulsion was prepared by mixing 175 μg Tween 20, 155 μL linoleic acid, and 0.04 M potassium phosphate buffer (pH 7.0). A 1 mL of sample at different concentrations in 99.5% ethanol was mixed with 4.1 mL linoleic emulsion, 0.02 M phosphate buffer (pH 7, 8 mL) and distilled water (7.9 mL). The mixed solutions of all samples (21 mL) were incubated in screw cap-tubes under dark conditions at 40 ℃ at certain time intervals. To 0.1 mL of this mixture was pipeted and added with 9.7 mL of 75% and 0.1 mL of 30% ammonium thiocyanate sequentially. After 3 min, 0.1 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture. The peroxide level was determined by reading daily of the absorbance at 500 nm in a spectrophotometer (Schimadzu UV/Vis-240IPC). All test data was the average of three replicate analyses. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

% Inhibition = $[(A_0 - A_1) / A_0] \times 100$ (1)

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of extracts or standard compounds.

2.4. Statistical analysis

Conventional statistical methods were used to calculate means and standard deviations of three simultaneous assays carried out with the different methods. Analysis of variance (ANOVA) was applied to data to determine differences ($P< 0.01$). ANOVAs with two factors (plant extract and concentration) were applied for each parameter.

3. Results

3.1. Phenolic compounds content

Phenolics are aromatic secondary plant metabolites, and are widely spread throughout the plant kingdom. Phenolics have been associated with color, sensory qualities, nutritional, and antioxidant properties[27]. The total phenolic content in six wild plants is given in (Figure 1).

The six plants were found to have widely varying phenolics levels, ranging from 1.1 g/100 g plant for *Achillia fragrantissima (A. fragrantissima)* to 5.6 g/100 g for *Artemisia monosperma (A. monosperma)*. All plants had high phenolics content and they have the following sequence, 1.08, 1.36, 2.92, 3.71, 3.9 and 5.658 g/100 g DW for *A. fragrantissima*, *Rhantarium epapposum (R. epapposum)*, *Anthemis deserti (A. deserti)*, *Pulicaria crispa (P. crispa)*, *Picri scyanocarpa (P. scyanocarpa)* and *A. monosperma*,

respectively that may be responsible for reproduction of high antioxidant activity.

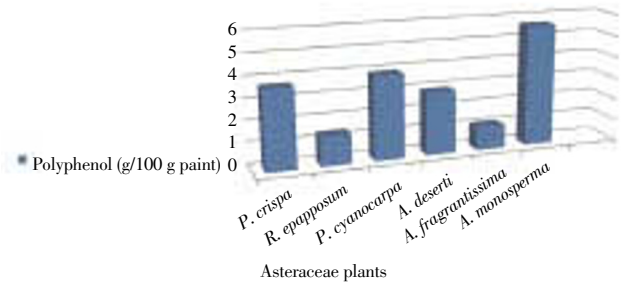

Figure 1. Total polyphenol content of different plants.
$P<0.01$.

3.2. Radical scavenging activity (DPPH)

A significant decrease ($P<0.01$) in the concentration of DPPH was observed due to the scavenging ability of tested extracts and standards (Figure 2). Standards, L-ascorbic acid and BHT, showed lower effect as compared to tested plant extracts at concentrations 25, 50 and 100 μg/mL while they have approximately the same results at the other two concentrations, 200 and 400 μg/mL. The minimum percentage of DPPH scavenging ability by all tested extracts at 25 μg/mL was the same of standards at 200 μg/mL, this activity was significantly increased with increasing extract concentration.

The scavenging ability, decolorization, at high concentration was in the following order: Ascorbic acid>*P. cyanocarpa*> *A. deserti*> *P. crispa*> BHT> *R. epapposum*, *A. fragrantissima*> *A. monosperma*.

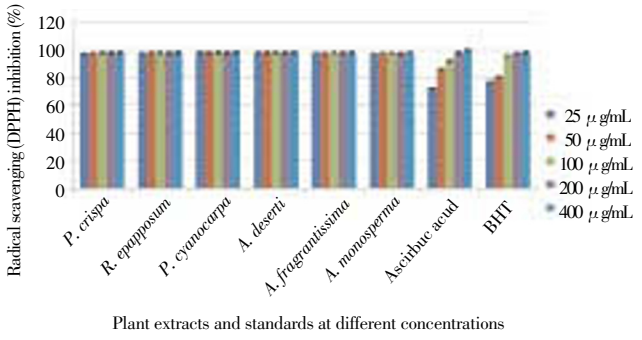

Figure 2. Free radical scavenging ability of different plant extracts and standards at tested concentrations.

3.3. Total reduction capability

The reduction capability of all tested plant extracts increased dependently with increasing concentration (Figure 3) and these increments were statistically significant ($P<0.01$). All tested extracts found to have lower reducing power as compared to standard materials (L-ascorbic and BHT) at all concentrations (25, 50, 100, 200 and 400 μg/mL). Reducing power of extracts and standards at high concentration followed the order: Ascorbic> BHT>*P. cyanocarpa*> *A. deserti*> *A. monosperma*> *P. crispa*> *R. epapposum*> *A. fragrantissima*, respectively.

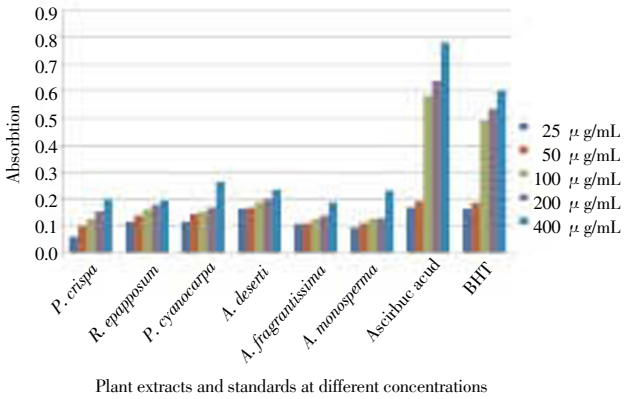

Figure 3. Total reductive capability of plant extracts and standards.
ANOVA one way followed by LSD was used, $n=3$, $P<0.01$.

3.4. Ferrous ion chelating effect

The purpose of the test of ferrous ion chelating effect was to determine the capacity of some Asteraceae plants to bind to the ferrous ion catalyzing oxidation. The ferrous ion chelating effect of *P. cyanocarpa*, *A. deserti*, *A. monosperma*, *P. crispa*, *R. epapposum*, *A. fragrantissima* and standards at different concentrations, 25, 50, 100, 200, and 400 μg/mL, is presented in (Figure 4). The presented data show that chelating effect was concentration dependent for all extracts. Tested plants extracts at the highest concentration (400 μg/mL) showed maximum chelating effect (100%) and they found to be more efficient than standards except *P. cyanocarpa* which showed the lowest effect (84.38%) at the same concentration. *A. fragrantissima* and *A. monosperma* were the potent ferrous ion chelator extracts at all concentrations; they showed 100% chelating effect at 100, 200 and 400 μg/mL.

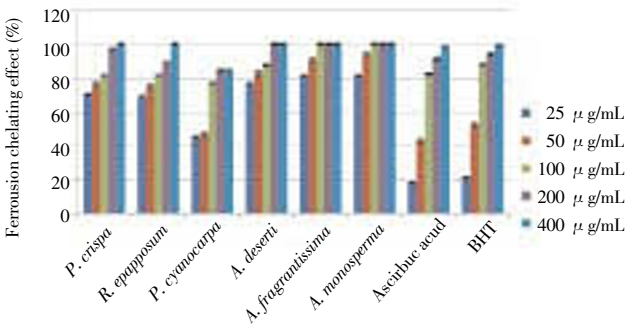

Figure 4. Ferrous ion chelating effect of some Asteraceae plants and standards at different concentration.
ANOVA one way was used, $n=3$, $P<0.01$.

3.5. Superoxide anion radicals scavenging activity

The inhibition percentage of superoxide radical generation by some Asteraceae plants extracts at different concentrations was determined and compared with BHT and L-ascorbic acid as standards at the same concentrations (Figure 5). All plant extracts showed 100% scavenging effect at 400 μg/mL while standards had 98% scavenging effect at the same concentration. Superoxide anion radical inhibition percentage was increased gradually with increasing extract conc., concentration dependent for all plant extracts and standards. *P. cyanocarpa* and *A. deserti* had the highest anion radical scavenging power at all concns. Plant extract inhibition percentages were arranged in the following order: *P. cyanocarpa* > *A. deserti* > *A. monosperma* > *A. fragrantissima* > BHT > *P. crispa* > *R. epapposum* > L-ascorbic acid, this order is true for all concentrations.

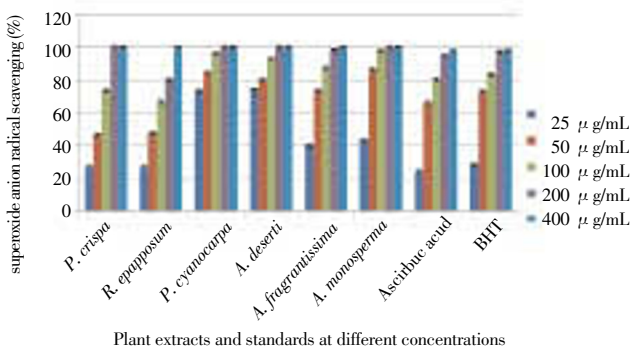

Figure 5. Superoxide anion radical scavenging activity of some Asteraceae plants and standards.
Data analyzed by ANOVA one way, n=3, P<0.01.

3.6. Total antioxidant capacity

The ABTS/H_2O_2 discoloration method is reported to represent the total antioxidant capacity of plant extracts. The total antioxidant capacity of some Asteraceae plants at different concentrations is presented in (Figure 6). *A. deserti* and *A. fragrantissima* showed remarkable antioxidant capacity as compared to other plants at 400 μg/mL (100% for two extracts) and they reached the same level of capacity at the same concentration. The antioxidant capacity was significantly enhanced by increasing extract concentration in a concentration dependent response for all extract. The lowest values were recorded for *P. crispa* at all concentration. All extract exhibited elevation in antioxidant capacity when their conc. was increased from 25 to 400 μg/mL. No significant difference was observed in total antioxidant capacity of *R. epapposum* when conc. was increased from 100 to 200 μg/mL also the same trend was observed for *A. monosperma* and ascorbic acid (from 200 to 400 μg/mL) and BHT (from 100 to 200 to 400 μg/mL).

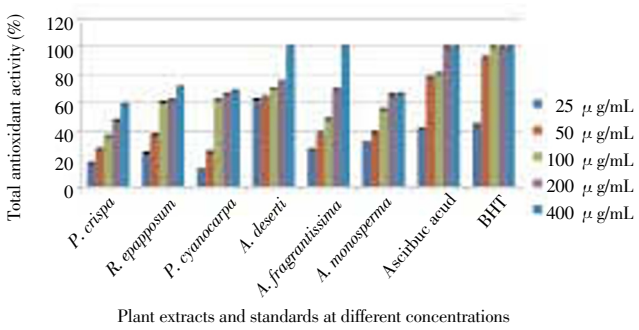

Figure 6. Total antioxidant activity of some Asteraceae plants and standards.
Data were analyzed by ANOVA one way, n=3, P<0.01.

3.7. Inhibition of lipid peroxidation

The TBARS assay is a sensitive method, requires small sample amounts and provides reproducible results. This method is preferable for obtaining useful data in an environment similar to the real-life situation and allows testing of both lipophilic and hydrophilic substances[28].

Lipid peroxidation was significantly inhibited by all plant extracts at all tested concentration with gradual inhibition percentage increments (Figure 7). The maximum inhibition percentage was presented with highest extract concentration. Plant extracts inhibition percentages at high concentration are in the following order; *A. fragrantissima* = *P. crispa* = *R. epapposum* > BHT > Ascorbic acid > *P.cyanocarpa* > *A. deserti* > *A. monosperma*. Both *A. fragrantissima* and *R. epapposumshowed* 100% lipid peroxidation inhibition at 200 and 400 μg/mL which is equally with BHT inhibition percentage. The same percentage was recorded with *P. crispaat* 400 μg/mL. Increasing concentration of *A. fragrantissima* and *R. epapposum* from 200 to 400 μg/mL didn't show any significant increment in inhibition percentage. Inhibition percentage was magnified by increasing concentration of *P. crispa*, *R. epapposum* and *P. cyanocarpa* from 100 to 200 μg/mL.

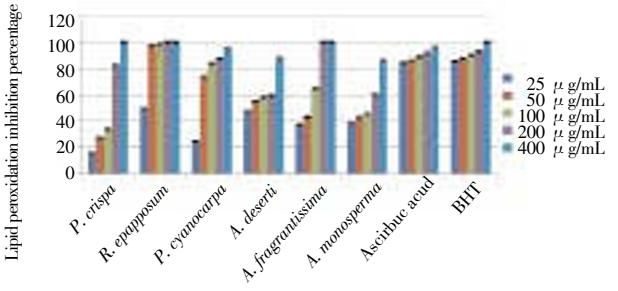

Figure 7. Lipid peroxidation inhibition percentage of some Asteraceae plants and standards.
Data were analyzed by ANOVA one way, n=3, P<0.01.

4. Discussion

The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging[29,30]. Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of these, total antioxidant activity, reducing power, DPPH assay, metal chelating, active oxygen species such as H_2O_2, $O_2^{\bullet -}$ and $OH^{\bullet}$ quenching assays are most commonly used for the evaluation of antioxidant activities of extracts[31-33].

The use of simplified model systems, which mimic the main features of a given food system, or antioxidant assays for quantifying the antioxidant action can be very helpful in clarifying the action of potential antioxidants[34]. The DPPH free radical does not require any special preparation and is considered a simple and very fast method for determining antioxidant activity. In contrast, DPPH can only be dissolved inorganic media, especially in ethanol, which is an important limitation when interpreting the role of hydrophilic antioxidants. The radical scavenging capacity of Asteraceae was tested using the 'stable' free radical, DPPH. *P. cyanocarpa* and *A. deserti* extracts are the most effective extracts as radical scavenger at all concentration to reach maximum and mimic standards activity at high concentration. The same extracts showed promising effect and the same trend of data as reducing agents and superoxide radical scavenger in concentration dependent manner.

One of the possible mechanisms of the antioxidative action is the chelation of transition metals. Metal chelating capacity is an important since; it reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh *et al*, 1999). It was reported that chelating agents, who form bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion[34]. Transition metal ions can stimulate lipid peroxidation at two ways: (a) participating in the generation of initiating species; and (b) accelerating peroxidation, decomposing lipid hydroperoxides into other components which are able to abstract hydrogen, perpetuating the chain of reaction of lipid peroxidation. Analysis of metal ion-chelating properties showed that all tested extracts at different concentrations showed potent effect as ferrous ion chelators, where *A. fragrantissima* and *A. monosperma* showed the promising effect as ion chelators, especially at high concentration, 200 and 400 μg/mL. Total antioxidant activity of all tested Asteraceae extracts was determined by the ABTS- peroxidase method. *Anthemis deserti* and *A. fragrantissima* exhibited effective antioxidant activity at all doses and the activity is a concentration dependent response.

Lipid peroxidation is important deteriorate reaction in food during storage and processing. It not only causes a loss in food quality but also is believed to be associated with some diseases such as carcinogenesis, mutagenesis, ageing, and arteriosclerosis. The role of active oxygen and free radicals in tissue damage in such diseases, are becoming increasingly recognized. Cancer, emphysema, cirrhosis, arteriosclerosis, and arthritis have all been correlated with oxidative damage[35]. Inhibition of lipid peroxidation by some Asteraceae plant extracts at different concentration using TBARS assay. All of them exhibited significant increments with increasing conc. *P. crispa, R.papposum* and *A. fragrantissima* presented 100% inhibition percentage which more than standards at the high dose (400 μg/mL), this lipid peroxidation inhibition may be attributed to their activities as radical scavenger, ion chelators, superoxide anion radical scavenger and hydrogen donors as well as their content of total phenolic content as mentioned through this study.

Active oxygen, either in the form of superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), or singled oxygen($1O_2$), is a product of normal metabolism and attacks biological molecules, leading to cell or tissue injury. When the mechanism of antioxidant protection becomes unbalanced by exogenous factors such as smoking, ionising radiation, certain pollutants, organic solvents and pesticides and endogenous factors such as normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes may occur, resulting in above-mentioned diseases and accelerating ageing[36]. However, antioxidant supplements or foods rich in antioxidants may be used to help the human body in reducing oxidative damage by free radicals and active oxygen[37,38]. This study showed that plant extracts under investigation may be used as potent agents in reducing oxidative stress.

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Conflict of interest statement

We declare that we have no conflict of interest

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