

## Antioxidant capacity and polyphenolic content of seven Saudi Arabian medicinal herbs traditionally used in Saudi Arabia

Abdelaaty A Shahat<sup>1,2\*</sup>, Abeer Y Ibrahim<sup>3</sup> & Mansour S Alsaied<sup>1</sup>

<sup>1</sup>Pharmacognosy Department, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia;

<sup>2</sup>Phytochemistry Department, <sup>3</sup>Medicinal and Aromatic Plants Research Department.,

National Research Centre, El-Bohous Street, 12311, Dokki, Egypt

E-mails: aashahat@hotmail.com; ashahat@ksu.edu.sa

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The using of plants of Saudi Arabia for the cure of many ailments is ancient and still available among the tribal and local people and medicinal healers. In this study, alcoholic extract of seven plants from Tanhat protected area, Saudi Arabian, were studied for their antioxidant properties. The antioxidant properties were evaluated using different antioxidant tests, including inhibition of lipid peroxidation, reducing power, metal ion chelation, scavenging of free radical, superoxide anion radical, hydrogen peroxide and nitric oxide as well as total antioxidant capacity. *Trigonella hamosa* and *Ducrosia anethifolia* had powerful antioxidant activity, had 100% inhibition on peroxidation of linoleic acid emulsion at 200 µg/ml while *Cleome ambliocarpa* was the most efficient as scavenger. The presence of phenolic compounds and flavonoids in the extracts, which were detected by Folin-Ciocalteu may be responsible for the antioxidant activities of *Heliotropium ramosissimum* which exhibited the highest reduction capability with highest polyphenolic content (48mg/100mg extract as chlorogenic acid). Moreover, *Trigonella hamosa* and *Convolvulus prostates* had effective metal chelating activity (100% ion chelation at 100µg/ml). Those various antioxidant activities were compared to standard antioxidants such as butylatedhydroxytoluene (BHT) and vitamin C.

**Keywords:** Antioxidant, Traditional medicinal plants, Lipid peroxidation

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Medicinal plants have been recognized for their therapeutic benefits for centuries and continue to attract increasing attention because of their potential benefits especially in the field of medicine and pharmacology. Medicinal plants constitute an important source of new candidates for therapeutic compounds, in regards to the chemical diversity found in several species<sup>1</sup>. By now people have started to look for high-quality dried herbal products that are closely associated with the quality of common raw herbal materials. Reactive oxygen species (ROS) production occurs during normal cell metabolism, both in animals and plants. Excess of ROS leads to oxidative stress, resulting in oxidative DNA damage which is implicated in the pathogenesis of numerous disorders, e.g. cardiovascular, atherosclerosis, reperfusion injury, cataract genesis, rheumatoid arthritis, inflammatory disorders and cancer. Plants contain many different dietary nutrients

possess strong antioxidant capacities, such as phenolic compounds and vitamins<sup>2</sup>.

Many plants have been identified as having potential antioxidant activities and their consumption recommended<sup>3,4</sup>. The current focus is toward natural antioxidants, especially plant polyphenolics. It is interested to investigate the antioxidant properties of herbals especially those traditionally used in folk medicine, *Teucrium olivarianum*, *Echium arabicum*, *Ducrosia anethifolia*, *Heliotropium ramosissimum*, *Cleome ambliocarpa*, *Trigonella hamosa* and *Convolvulus prostates*. These plants are used in folk medicine as a remedy for liver disorders, an analgesic and pain reliever for headache, backache, colic and colds, brain related disease; improve memory, skin disease and other disease<sup>1,3</sup>. The aim of the present study is to examine the total phenolic content and related total antioxidant potential metal chelator and as inhibitor for lipid peroxidation by different assays in seven medicinal plants used traditionally in the Kingdom of Saudi Arabia.

\*Corresponding author

## Methodology

### Plant collection and authentication

All plants (Table 1) were collected from the Tanhat protected area, 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.

### Plant extract preparation

The aerial parts of plants were collected and dried under shade. The dried samples were powdered and used for solvent extraction. 200 gm of dried sample was extracted twice with 600 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°C. The dry extract obtained with each solvent was weighed.

### Free radical scavenging effect

The free radical scavenging activity was measured at different concentrations (25, 50, 100, 200 and 400 µg/ml) by 1,1-diphenyl-2-picryl-hydrazil (DPPH•) using the method of Yamaguchi *et al.*<sup>11</sup>. The DPPH• radical concentration in the reaction medium was calculated from the following equation: DPPH• scavenging effect (%) =  $100 - [(A_0 - A_1) / A_0] \times 100$ , Where  $A_0$  was the absorbance of the control reaction and  $A_1$  was the absorbance in the presence of the sample of polysaccharide.

### Reduction capability

The reduction capability of plant extracts and standards was determined according to the method of Oyaizu<sup>12</sup>. The different concentrations (25, 50, 100, 200 and 400 µg/ml) in 1ml of methanol were 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°C for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture, which was then centrifuged for 10 min at  $1000 \times g$  (MSE Mistral 2000, UK, and Serial No.: S693/02/444). The upper layer of solution (2.5ml) was mixed with methanol (2.5ml) and  $FeCl_3$  (0.5ml, 0.1%), and the absorbance was measured at 700nm in a spectrophotometer (Jasco V630 Serial No. C317961148). Vitamin C and BHT were used as controls. Higher absorbance of the reaction mixture indicated greater reducing power.

### Metal chelating effect

The chelating of ferrous ions by the plant extracts and standards was estimated by the method of Dinis *et al.*<sup>13</sup>. The percentage of inhibition of ferrozine- $Fe^{2+}$  complex formation was given by the formula: Inhibition (%) =  $[(A_0 - A_1) / A_0] \times 100$ , Where  $A_0$  was the absorbance of the control, and  $A_1$  was the absorbance in the presence of the sample and standards. The control contains  $FeCl_2$  and ferrozine.

### Superoxide anion scavenging effect

Measurement of superoxide anion scavenging effect of plant extracts was based on the method described by Liu *et al.*<sup>16</sup> with slight modifications. 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, and  $A_1$  was the absorbance of samples or standards.

### Scavenging of hydrogen peroxide

The ability of plant extracts and standards to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*<sup>15</sup>. The percentage of scavenging of hydrogen peroxide of

Table 1—Medicinal plants used in the present study

Plant species (Voucher No)	Family	Traditional use	(Yield in %)
1 <i>Teucrium oliverianum</i> (15930)	Lamiaceae	Antinociceptive effect <sup>6</sup> , antioxidant activity and antimicrobial activities	(20 %)
2 <i>Echium arabicum</i> (15931)	Boraginaceae	Antiplasmodial and antitrypanosomal activity <sup>7</sup>	(14.5 %)
3 <i>Ducrosia anethifolia</i> (15937)	Apiaceae	Analgesic and pain reliever for headache, ackache, colic, and colds <sup>8</sup> .	(26 %)
4 <i>Heliotropium ramosissimum</i> (15938)	Boraginaceae	Treatment of gout, rheumatism, and as anti-inflammatory and healing agents <sup>9</sup> .	(2.58 %)
5 <i>Cleome ambliocarpa</i> (15945)	Cleomaceae	Stomachics, rubefacients and in the treatment of scabies, rheumatic, fever, inflammation and a hypoglycemic agent <sup>10</sup> ,	(14.9 %)
6 <i>Trigonella hamosa</i> (15951)	Papilionaceae	a condiment and seasoning in food preparations and hypoglycemic	(22 %)
7 <i>Convolvulus prostates</i> (15953)	Convolvulaceae	Brain related disease, improve memory, skin disease <sup>11</sup>	(15.3 %)

extracts and standard compounds was calculated using the following equation:  $H_2O_2$  (%) =  $[(A_0 - A_1) / A_0] \times 100$ , Where  $A_0$  was the absorbance of the control and  $A_1$  was the absorbance in the presence of the samples and standards.

#### Nitric oxide radical scavenging effect

$NO^{\bullet}$  radical scavenging effect of extracts was determined by using a sodium nitroprusside (SNP) generating  $NO^{\bullet}$  system.  $NO^{\bullet}$  generated from SNP in aqueous solution at physiological pH reacts with oxygen to produce nitrite ions which were measured by the Greiss reagent which constitutes 1% Sulfanilamide in 5% ortho- $H_3PO_4$  and 0.1 % Naphthyl ethylene diaminedihydro chloride. The reaction mixture (2ml) containing various concentrations of the test polysaccharide and standard compounds and SNP (final concentration, 10mM) in phosphate buffered saline (PBS) pH 7.4 were incubated at 25°C for 150 min. After incubation, 1ml samples of reaction mixtures were removed and diluted with 1ml Greiss reagent. The absorbance of these solutions was measured at 540nm against the corresponding blank solution.

#### Total antioxidant capacity

Total antioxidant capacity was measured according to the method described by Miller and Rice-Evans<sup>16</sup>. Exactly 0.2 ml of peroxidase (4.4 units/ ml), 0.2ml of  $H_2O_2$  (50  $\mu$ M), 0.2 ml of ABTS (2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid, diammonium salt, 100 $\mu$ M) and 1ml methanol were mixed, and were kept in the dark for 1hr to form a bluish green complex after adding of 1ml plant extracts of different concentrations or VC and BHT, used as a control. All were tested in triplicates. The absorbance at 734nm was measured to represent the total antioxidant capacity and then was calculated as follows: Total antioxidant activity (%) =  $[1 - (A_{\text{sample}} / A_{\text{control}})] \times 100$ .

#### Lipid Peroxidation by linoleic assay

Inhibition of lipid peroxidation of plant extracts and standards was determined according to the method of Gülçin *et al.*<sup>17</sup> with some modifications. A pre emulsion was prepared by mixing 175  $\mu$ g Tween 20, 155  $\mu$ L linoleic acid, and 0.04M potassium phosphate buffer (pH 7.0). A 1 mL of sample in 99.5% ethanol was mixed with 4.1 mL linoleic emulsion, 0.02 M phosphate buffer (pH 7.8) and distilled water (pH7.9). The mixed solutions of all

samples (21mL) were incubated in screw cap-tubes under dark conditions at 40°C at certain time intervals. To 0.1mL of this mixture was pipetted and added with 9.7mL of 75% and 0.1mL of 30% ammonium thiocyanate sequentially. After 3 min, 0.1 mL of 0.02M ferrous chloride in 3.5% HCl was added to the reaction mixture. The peroxide level was determined by reading daily of the absorbance at 500 nm in a spectrophotometer. The same assay for VC and BHT was also determined for comparison. All test data was the average of three replicate analyses. The inhibition of lipid peroxidation percentage was calculated by the following equation: 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 extracts or standard compounds.

#### Determination of total phenolic compounds

Total soluble phenolic compounds in seven tested plant extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton<sup>18</sup> using chlorogenic acid as a standard phenolic compound. The total concentration of phenolic compounds in different extracts was determined as microgram of chlorogenic acid equivalent by using an equation that was obtained from standard chlorogenic acid graph:

$$\text{Absorbance} = 0.0053 \times \text{total phenols} \\ [\text{chlorogenic equivalent } (\mu\text{g}) - 0.0059].$$

#### Statistical analysis

In all cases analyses were performed in triplicate and data were averaged over the three measurements. The standard deviation (SD) was also calculated. Data were treated for multiple comparisons by analysis of variance (ANOVA), followed by the Duncan's Multiple Range test with significance level  $P < 0.01$  to determined significant differences between means.

## Results

#### Radical scavenging effect

All tested plant extracts showed potential scavenging effect against DPPH radicals. They exhibited low  $IC_{50}$  values, 15.05, 15.07, 15.22, 15.11, 14.09, 14.25 and 14.46 for *T. olivarianum*, *E. arabicum*, *D. anethifolia*, *H. ramosissimum*, *C. ambliocarpa*, *T. hamosa* and *C. prostates*, respectively that are nearly the same of those standards 17.29 and 16.25 for BHT and Vitamin C, respectively.

Table 2—Radical scavenging effect of plant extracts and standards at different concentrations

Sample	<i>T. livarianum</i>	<i>E. arabicum</i>	<i>D. anethifolia</i>	<i>H. ramosissimum</i>	<i>C. ambliocarpa</i>	<i>T. hamosa</i>	<i>C. prostates</i>	BHT	Vitamin C
25µg/ml	83.04±1.11	82.92±1.74	82.12±2.07	82.75±1.88	88.73±1.08	87.67±1.67	86.42±1.23 <sup>f</sup>	72.28±2.11	76.91±2.75
50µg/ml	92.17±1.52	91.22±1.67	90.92±1.95	90.05±1.09	92.62±1.24	90.84±1.58 <sup>e</sup>	88.58±1.43 <sup>f</sup>	86.17±2.15	80.46±1.11
100µg/ml	95.18±1.31	94.26±1.69	93.14±1.83	93.26±1.07	96.31±1.45 <sup>d</sup>	92.96±1.94 <sup>e</sup>	91.69±1.56	92.31±1.89	94.18±2.17
200µg/ml	98.18±1.84 <sup>a</sup>	96.31±2.10 <sup>b</sup>	96.22±1.36 <sup>c</sup>	96.35±2.11	98.43±2.71 <sup>d</sup>	95.14±1.37	95.23±1.98	98.28±1.37	97.88±0.96 <sup>e</sup>
400µg/ml	98.31±2.15 <sup>a</sup>	98.31±2.84 <sup>b</sup>	98.39±2.84 <sup>c</sup>	100±1.02	100±2.06	100±1.78	100±2.08	100±2.64	98.32±0.99 <sup>e</sup>
IC <sub>50</sub> (µg/ml)	15.05	15.07	15.22	15.11	14.09	14.25	14.46	17.29	16.25

Data are presented as mean of triplicates± standard deviation. Data were analyzed by ANOVA one way followed with Post Hoc for multiple comparisons. BHT is butylatedhydroxytoluene. Groups have the same letter mean no significant difference between them

Table 3—Plant extracts and standards as Metal ion chelator

Sample	<i>T. olivarianum</i>	<i>E. arabicum</i>	<i>D. anethifolia</i>	<i>H. ramosissimum</i>	<i>C. ambliocarpa</i>	<i>T. hamosa</i>	<i>C. prostates</i>	BHT	Vitamin C
25µg/ml	65.63±2.14	62.5±2.41	73.44±1.33	78.13±1.94	64.07±1.35	81.25±1.77	84.38±2.43	21.22±1.32	18.18±1.94
50µg/ml	75±1.98	70.31±1.58	78.13±1.84	82.28±2.51 <sup>c</sup>	71.8±1.59	90.63±1.58	97.11±2.71	52.41±1.22	43.15±2.62
100µg/ml	81.25±1.77	75±1.64 <sup>a</sup>	82.82±2.45 <sup>b</sup>	85.94±2.11 <sup>c</sup>	84.38±1.78 <sup>d</sup>	100±1.94	100±1.49	87.93±3.14	82.33±2.48
200µg/ml	85.94±2.31	79.69±1.72 <sup>a</sup>	84.38±2.71 <sup>b</sup>	90.63±1.76	85.94±1.82 <sup>d</sup>	100±1.11	100±1.75	94.16±2.51	91.52±1.79
400µg/ml	95.32±1.64	85.44±1.48	89.07±1.86	100±1.69	100±1.93	100±2.41	100±1.66	99.09±2.71	98.19±1.95
IC <sub>50</sub> (µg/ml)	19.05	20	17.02	15.99	19.51	15.38	14.82	58.91	68.76

Data are presented as mean of triplicates± standard deviation. Data were analyzed by ANOVA one way followed with Post Hoc for multiple comparisons. BHT is butylatedhydroxytoluene. Groups have the same letter have no significant difference between them.

Data presented in Table 2 showed significant concentration dependent increments for all plant extracts and standards except increments at 200 and 400µg/ml for *T. olivarianum*, *E. arabicum*, *D. anethifolia* and VC were insignificant, the same trend of data was observed for *C. ambliocarpa* at 100 and 200 µg/ml, *T. hamosa* at 50 and 100 µg/ml and *C. prostates* at 25 and 50 µg/ml.

### Reduction capability

Fig.1 shows the reductive capabilities of plant extracts compared to BHT and VC. For the measurements of the reductive ability, we investigated the Fe<sup>3+</sup>–Fe<sup>2+</sup> transformation in the presence of plant extracts using the method of Oyaizu<sup>12</sup>. The reducing power of extracts increased concentration dependently. All of the concentrations showed lower activities than the control in a statistically significant ( $P < 0.05$ ) manner.

There is no significant differences was observed with increasing conc. from 50 to 100 µg/ml, this trend is true with all plant extracts except in case of *H. ramosissimum* and *T. hamosa*. The plant extracts were arranged in the following order according to their reduction capability at 400 µg/ml; BHT>VC >*H. ramosissimum*>*C. prostates*> *T. olivarianum*> *E. arabicum*> *T. hamosa*> *D. anethifolia*> *C. ambliocarpa*.

### Metal ion chelation effect

As shown in Table 3, the formation of the Fe<sup>2+</sup>-ferrozine complex was not completed in the presence of plant extracts, indicating that these extracts chelate the iron. The absorbance of Fe<sup>2+</sup>-ferrozine complex was linearly decreased dose-dependently (from 25 to 400 µg), that is true for all plant extracts. The difference between extracts and the controls were statistically significant ( $P < 0.01$ ). The percentages of metal chelating capacity of 400µg/ml reached 100% for *H. ramosissimum*, *C. ambliocarpa*, *T. hamosa* and *C. prostates* while BHT and VC produced 99 and 98% iron scavenging percentage, respectively. *T. hamosa* and *C. prostates* showed 100% chelation activity with a concns 100, 200 and 400 µg/ml. *E. arabicum* showed the lowest chelation effect for iron(IC<sub>50</sub>= 20 µg/ml).

### Scavenging of super oxide radicals, nitric oxide and hydrogen peroxide as well as total antioxidant capacity of plant extracts

In the PMS–NADH–NBT system, superoxide anion derived from dissolved oxygen by PMS–NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Table 4 shows IC<sub>50</sub> values calculated as

Table 4—Plant extracts as scavenger and their total antioxidant capacity

Plant extracts and tested references	IC <sub>50</sub> (µg/ ml)			
	Superoxide anion radical scavenging	Nitric oxide radical scavenging	Hydrogen peroxide scavenging	Total antioxidant capacity
<i>T. olivarianum</i>	37.50	15.19	15.59	19.62
<i>E. arabicum</i>	17.06	6.94	7.09	22.56
<i>D. anethifolia</i>	17.05	6.91	7.07	18.34
<i>H. ramosissimum</i>	26.78	10.85	11.13	17.68
<i>C. ambliocarpa</i>	12.51	5.06	5.91	12.50
<i>T. hamosa</i>	31.25	12.76	13.12	25.82
<i>C. prostatus</i>	62.50	25.63	25.57	19.24
BHT	43.83	17.75	15.59	28.35
Vitamin C	51.80	17.94	15.17	30.48

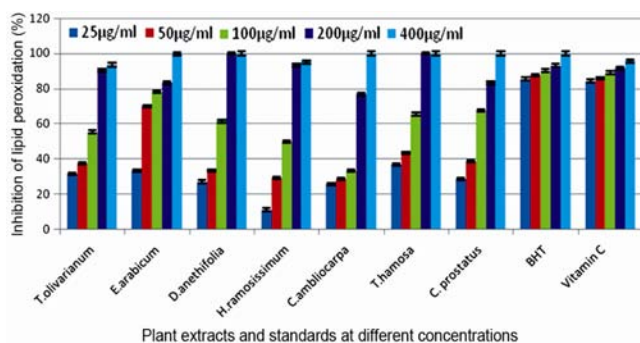


Fig. 1—Reduction capability of tested plant extracts and standards at different concentrations. Data are mean of triplicates. Data were analyzed using ANOVA one way

the concentration required for inhibition of superoxide radical generation by 50%. The *C. ambliocarpa* extract exhibited higher superoxide radical scavenging activity (12.51 µg/ ml) while *C. prostatus* showed the lowest values (62.5 µg/ ml) in this concern. Extracts and standards was arranged in the following order according to their IC<sub>50</sub>, *D. anethifolia* > *E. arabicum* > *H. ramosissimum* > *T. hamosa* > *T. olivarianum* > BHT > VC.

The compound disodium pentanonitrosyl ferrate (2-) dehydrate which was used as a nitric oxide donor decomposed at a physiological pH (7.2) and generated nitric oxide which under aerobic condition produced stable nitrite. The griess assay used to determine the nitrite concentration is a suitable method for bio-guided fractionation of potential anti-inflammatory plant extracts. Nitric oxide radical generated by sodium nitroprusside at physiological pH was found to be inhibited by tested plant extracts at all tested concentrations (25, 50, 100, 200 and 400 µg/ ml) in a dose dependent manner, the highest concentration the highest inhibition percentage. *C. ambliocarpa* possess

the highest inhibition percentage (IC<sub>50</sub>= 5.06 µg/ ml) followed with *D. anethifolia* and *E. arabicum* (IC<sub>50</sub>=6.91 and 6.94 µg/ ml, respectively). On the other hand, the lowest mean value was recorded with *C. prostatus* (IC<sub>50</sub>= 25.63 µg/ ml). The other tested extract and standards had the following order; *H. ramosissimum* > *T. hamosa* > *T. olivarianum* > BHT > VC.

The ability of plant extracts to scavenge H<sub>2</sub>O<sub>2</sub> was determined according to the method of Ruch *et al.*<sup>15</sup>. The scavenging ability of extracts on H<sub>2</sub>O<sub>2</sub> is shown in Table 4 and compared with BHT and VC as standards. All tested plant extracts were capable of scavenging H<sub>2</sub>O<sub>2</sub> in a dose-dependent manner. All plant extracts were superior to standards at all concentrations therefore, they exhibited low IC<sub>50</sub> except *C. prostates* that showed the lowest IC<sub>50</sub> (25.57 µg/ ml) and there were statistically a very significant correlation between those values and standards compounds (*P* < 0.01). *C. ambliocarpa*, *D. anethifolia* and *E. arabicum* extracts possess potential H<sub>2</sub>O<sub>2</sub> scavenging ability (IC<sub>50</sub>= 5.91, 7.07 and 7.09 µg/ ml, respectively) followed with *H. ramosissimum* > *T. hamosa* > *T. olivarianum* > BHT > VC. The ABTS/H<sub>2</sub>O<sub>2</sub>/HRP decoloration method is reported to represent the total antioxidant activity of natural extracts. The plant extracts concentrations that produce 50% ABTS discoloration (IC<sub>50</sub>) were presented in Table 4. All plant extracts exhibited high antioxidant activity more than standards BHT and VC (IC<sub>50</sub>= 28.35 and 30.48, respectively). *C. ambliocarpa* was the most efficient one (IC<sub>50</sub>=12.5) in ABTS discoloration while the lowest efficient extracts were *T. hamosa* and *E. arabicum* (25.82 and 22.56, respectively). *H. ramosissimum*, *D. anethifolia*, *C. prostates* and

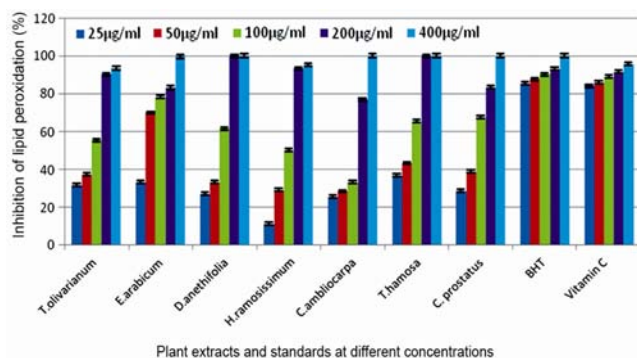


Fig. 2—Inhibition of linoleic acid peroxidation by plant extracts and standards at different concentrations. Data are presented as mean of triplicates  $\pm$  SD

*T. olivarianum* extracts showed nearly the same effect with insignificant differences, the  $IC_{50}$  values were 17.68, 18.34, 19.24 and 19.62, respectively.

### Inhibition of lipid peroxidation

Effect of plant extracts as lipid peroxidation inhibitors was evaluated by linoleic assay. All tested plant extracts inhibited lipid peroxidation in a concentration dependent manner. Inhibition of lipid peroxidation was increased significantly and gradually with increasing concentration, Fig. 2. *T. hamosa* and *D. anethifolia* inhibited lipid peroxidation of linoleic acid by 100 at 200  $\mu$ g/ml while *C. ambliocarpa*, *C. prostates*, and *E. arabicum* reached 100% inhibition percentage at 400  $\mu$ g/ml as BHT. On the other hand, *H. ramosissimum* produced the same activity level of VC at 400  $\mu$ g/ml (95% inhibition percentage), where *T. olivarianum* extract showed the lowest effect in this concern.

### Polyphenol content

Polyphenol content of plant extracts was determined by the method of Slinkard and Singleton<sup>18</sup>. Plant extracts showed different levels of polyphenolics, they were ranged from 10% to 48% as compared to plant extract. *H. ramosissimuma* had the highest polyphenolic compounds content determined as chlorogenic acid (48 gm/100 gm plant extract) (Fig. 3) while the minimum percentage of polyphenolic compounds was that of *C. prostates* (10%). Plant extracts was arranged in the following order according to their content of polyphenolics; *C. prostates* > *C. ambliocarpa* > *T. olivarianum* > *T. hamosa* > *D. anethifolia* > *E. arabicum* > *H. ramosissimum*.

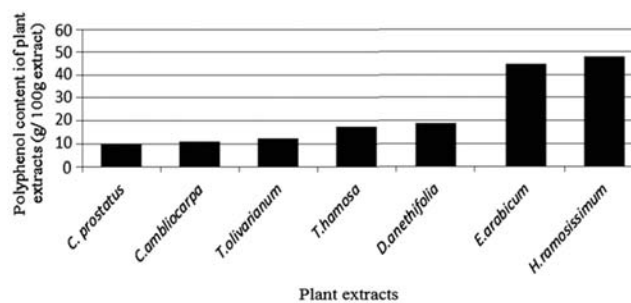


Fig. 3—Polyphenolic content of plant extracts evaluated as chlorogenic acid. Data are presented as mean of triplicates  $\pm$  SD

### 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. It was reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic conditions such as arteriosclerosis. 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. Based on the data obtained from the present study, all tested plant extracts exhibited free radical inhibitor or scavenger activity. *H. ramosissimum*, *C. ambliocarpa*, *T. hamosa* and *C. Prostates* were scavenged DPPH radicals by 100%. This results means this extracts may role as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in the human body.

The reducing capacity of seven medicinal plants was measured by evaluating  $Fe^{3+}$ - $Fe^{2+}$  conversion. The reducing capacity of extract or compounds may serve as an important indicator of its potential antioxidant activity. *H. ramosissimum* and *T. hamosa* were the most efficient extracts in reducing ferric ion while other extracts produced low activity which indicates that the two extracts may play as hydrogen donor.

Ferrous ion, commonly found in food systems, is well known as an effective pro-oxidant. Metal chelating capacity is important since it reduced the

concentration of the catalyzing transition metal in lipid peroxidation. The purpose of ferrous ion chelating activity test is to determine the capability of testing material to bind the oxidation catalytic ferrous ion. *T. hamosa* and *C. prostates* were the most effective extracts as metal ion chelator. They inhibited formation of the  $\text{Fe}^{2+}$ -ferrozine complex by 100% at 100  $\mu\text{g}/\text{ml}$ , however, *H. ramosissimum* and *C. ambliocarpa* chelated ions by 100% at 400  $\mu\text{g}/\text{ml}$ . These data demonstrate a marked capacity for iron binding of *T. hamosa* and *C. prostates* which suggesting that their action as peroxidation protector may be related to its iron binding capacity through reduction of catalyzing transition metal concentration. It was reported that chelating agents, which 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. Reactive oxygen species and reactive nitrogen species (RNS, e.g. nitric oxide,  $\text{NO}\cdot$ ) are well recognized for playing a dual role as both deleterious and beneficial species<sup>19</sup>. Superoxide anion is one of the most representative free radicals. In cellular oxidation reaction, superoxide radicals are normally formed first, however, their effects can be magnified because they produce other kinds of cell damaging free radicals and oxidizing agents. *C. ambliocarpa* found to be supereminent extract in scavenging superoxide anions in PMS-NADH-NBT system ( $\text{IC}_{50}= 12\mu\text{g}/\text{ml}$ ) followed with *E. arabicum* and *D. anethifolia*.  $\text{NO}\cdot$  is generated in biological tissues by specific nitric oxide synthases (NOSs), which metabolizes arginine to citrulline with the formation of  $\text{NO}\cdot$  via a five electron oxidative reaction<sup>20</sup>. Overproduction of reactive nitrogen species is called nitrosative stress which may occur when the generation of reactive nitrogen species in a system exceeds the system's ability to neutralise and eliminate them. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function. The present results indicate the potent effect of *C. ambliocarpa* as scavenger of nitric oxide radicals in Griess system followed with *E. arabicum* and *D. anethifolia*. Reactive nitrogen intermediates, such as nitric oxide ( $\text{NO}\cdot$ ), peroxynitrite ( $\text{ONOO}\cdot$ ) and nitrogen dioxide ( $\text{NO}_2$ ) has also been shown to play an important role in the inflammatory processes and possibly in carcinogenesis. The adoption of crude extracts of plants, such as infusions, for

self-medication by the general public, has arisen in the possibility that the impact of several diseases may be either ameliorated or prevented by improving the dietary intake of natural nutrients with antioxidant properties.

$\text{H}_2\text{O}_2$  is highly important because of its ability to penetrate biological membranes.  $\text{H}_2\text{O}_2$  itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. It is also clear that excessive production of free radicals causes damage to biological material and is an essential event in the etiopathogenesis of various diseases<sup>21</sup>. The production of these reactive species in healthy organism is approximately balanced by antioxidant defenses stems. However, an organism can be suffering from so called 'oxidative stress' while it is experiencing disturbance in the pro-oxidant- antioxidant balance in favor of the former, leading to potential damage. *C. ambliocarpa* found to have a powerful hydrogen peroxide scavenger which may play a role as protector from hydroxyl radical production in media containing excess amount of hydrogen peroxide by consumption of it.

It is well known that free radicals cause cell damage<sup>22</sup> through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury. Presence of unsaturated fatty acids in the lipid membranes, especially linoleic acid, makes them very susceptible to oxidative reactions. Inhibition of linoleic acid oxidation could be a good indication for antioxidant activity and has been widely used<sup>26</sup>. In this study extracts of seven plant species were evaluated for their anti-lipid peroxidation effect against linoleic acid in the reaction mixture. Linoleic acid peroxidation was determined spectrophotometrically. *T. hamosa* and *D. Anethifolia* inhibited linoleic peroxidation by 100% at 200  $\mu\text{g}/\text{ml}$  while *C. ambliocarpa*, *C. prostates* and *E. arabicum* reached 100% inhibition at 400ppm as BHT, reference compound. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. According to the recent reports, a highly positive relationship between total phenols and antioxidant activity was found in many plant species<sup>13</sup>. Phenolic compounds were determined in all tested extracts and *H. ramosissimum* had the highest content (48mg/100mg plant extract as chlorogenic acid equivalents) and the lowest one was *C. prostates* (10mg/100mg extract). The phenolic compounds may contribute directly to

the antioxidative action. It is suggested that polyphenolic compounds may have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 gm daily are ingested from a diet rich in fruits and vegetables. In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation. The antioxidant activity of tested extracts may be attributed to their phenolics. On the basis of the results of this study, it is clearly indicated that the seven tested plant extracts have strong antioxidant properties but *C. ambliocarpa* and *H. ramosissimum* are the most powerful antioxidant agents against various oxidative systems *in vitro*; moreover, they can be used as accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. The various antioxidant mechanisms of *H. ramosissimum* may be attributed to strong hydrogen donating ability as its high content of phenolics. *C. ambliocarpa* effectiveness as scavengers of hydrogen peroxide, superoxide, nitric oxide and free radicals may also attribute to phenolic compounds which may appear to be responsible for the antioxidant activity.

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