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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

The Antioxidant Activity and Polyphenolic Contents of Different Plant Seeds Extracts

Omar M. Atrooz

Department of Biological Sciences, Mutah University, P.O. Box 7, Jordan

Abstract: Different plant seeds extracts of *Citrus sinensis*, *Hordeum sativum*, *Triticum sativum*, *Canna indica*, *Citrullus vulgaris* and *Capsicum annum* were evaluated for their antioxidant activity by the following methods: 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging, reducing power, RBCs hemolysis and linoleic acid oxidation, along with the determination of total phenolic and flavonoids contents. All the methanolic extracts showed high antioxidant activity and have high contents of phenolic and flavonoid. The *Canna indica* extract exhibited strong antioxidant activity as a reducing power and as DPPH radical-scavenging (3.61 absorbance, 87.12%, respectively), while the *Hordeum sativum* extract exhibited highest inhibitory effect on RBCs hemolysis (59.55%) and the *Capsicum annum* extract has highest inhibitory effect on linoleic acid peroxidation (65.06%).

Key words: Total polyphenol contents, antioxidant activity, DPPH, flavonoids, seeds extracts

INTRODUCTION

Plants contain different natural products, which have a remarkable role in the traditional medicine in different countries. Nowadays, the prevention of many diseases has been associated with the ingestion of different plants rich in natural antioxidants (Johnson, 2001; Virgili *et al.*, 2001). It was found that a higher intake of such compounds is associated with a lower risk of mortality from different diseases (Ajih and Janardhanan, 2002; Lim *et al.*, 2002; McCune and Johns, 2002; Tziveleka *et al.*, 2002).

The protective effects of plant products are due to the presence of several components, such as enzymes, proteins, vitamins (Halliwell, 1996; Head, 1998), carotenoids (Edge *et al.*, 1997), flavonoids (anthocyanins) (Zhang and Wang, 2002) and other phenolic compounds (Sanchez-Moreno *et al.*, 1998; Argolo *et al.*, 2004).

Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, acting as oxygen scavengers (Shahidi and Wanasundara, 1992; Concepcion *et al.*, 1999) and prevent lipid auto oxidation (Brand-Williams *et al.*, 1995; Bondet *et al.*, 1997).

Natural antioxidants like vitamin C and E, carotenoids and polyphenols like flavonoids, are considered to be beneficial components from fruit and vegetables (Gupta and Prakash, 2009; Serteser *et al.*, 2009). They are responsible for the protective effects against different diseases (Peto *et al.*, 1981; Block *et al.*, 1992; Diplock, 1996; Rietjens *et al.*, 2002).

Phenolic compounds are extremely diverse: phenolic diterpenes (carnosol and rosmanol) (Houlihan *et al.*,

1984), isoflavone glycosides (in soybean) (Naim *et al.*, 1973; Eldridge and Kwolek, 1983) and several phenolic acids (chlorogenic, caffeic and ferulic acids) (Pratt and Birac, 1979). They are naturally present in essentially all plant materials, in vegetables, cereals, fruits, nuts and in plant products, such as wine, cider, beer, tea and cocoa (Bravo, 1998; Ramila *et al.*, 2005; Kelen and Tepe, 2007). The most important of the phenolics are the flavonoids. Several classes of flavonoid are differentiated on the degree of unsaturation and degree of oxidation of the three-carbon segment (Robards *et al.*, 1999).

The redox properties of phenolic compounds are the main factors for their antioxidant activity: reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelation potential (Parr and Bolwell, 2000; Atoui *et al.*, 2005).

In the present study, the antioxidant activity of different plant seeds extracts was evaluated by using widely accepted methods: DPPH radical-scavenging activity, reducing power, RBCs hemolysis and linoleic acid oxidation. The total phenolic and flavonoid will be determined by Delcour and de Varebeke (1985) methods, respectively.

MATERIALS AND METHODS

Different plant samples: *Citrus sinensis* (*C. sinensis*), *Hordeum sativum* (*H. sativum*), *Triticum sativum* (*T. sativum*), *Canna indica* (*C. indica*), *Citrullus vulgaris* (*C. vulgaris*) and *Capsicum annum* (*C. annum*) were purchased from local markets during summer, 2006 the seeds then isolated, grounded and the powder was kept at 4°C. This study was done at the research laboratories in Biological Department of Mutah University.

Chemicals: Methanol (Scharlau, Barcelona, Spain); HCl (Surechem product Ltd., England), Sodium carbonate (RIEDEL-Dehaen, Germany); gallic acid, p-Dimethylaminocinnamaldehyde, Catechin, 2,2'-azo-bis (2-methylpropionamidine)dihydrochloride (AAPH) and 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) (all from Sigma-Aldrich Chemie, Germany), Folin-Ciocalteu (KOCH-Light Ltd., Haverhill-Suffolk, England), FeSO₄.7H₂O (Fluka/Purum. Ph. Eur. Switzerland). Trichloroacetic acid (Janseen, Chimica, Eur. Pharma; USP, Belgium), Thiobarbituric acid (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were used.

Sample preparation: Forty grams of the seed powder Dry Weight (DW) were extracted once by using 70% methanol. The extracted materials were evaporated till dryness by rotary evaporator (RV 05-ST Janke and Kunkel, IKA, Germany).

Determination of total polyphenols: The total phenolic of the seed extracts was determined according to Folin-Ciocalteu method (Taga *et al.*, 1984). Briefly: 110 mg of seed extracts was dissolved in 25 mL of methanol, mixed by using vortex and 2 mL of this solution was added to 3 mL of 0.3% HCl. A 0.1 mL aliquot of the resulting solution was added to 2 mL of 2% Na₂CO₃ and after 2 min, 0.1 mL of Folin-Ciocalteu reagent (diluted with methanol 1:1) was added. The reaction mixtures were then incubated in the dark at 25°C for 30 min and then the absorbance was measured at 750 nm using Spectronic 1201 spectrophotometer (Milton Roy Company, USA). The concentration was calculated using gallic acid as standard and the results were expressed as milligram gallic acid equivalent per gram extract.

Determination of flavonoids: The determination of flavonoids was performed according to a colorimetric assay of Delcour and de Varebeke (Delcour and de Varebeke, 1985). One hundred milligrams of each extract was diluted in 10 mL of methanol and 1 mL of this solution was pipetted into a test tube. Five milliliter of the chromogen reagent (1.00 g of 4-dimethylaminocinnamaldehyde dissolved in cooled mixture of 250 mL concentrated HCl and 750 mL methanol, made up 1 L with methanol) were added to the extract solution and after 10 min the light absorbance was read at 640 nm by using Spectronic 1201 spectrophotometer against a blank with water instead of extract solution. A calibration curve was prepared with (±) catechin and the results were expressed as micrograms (±) catechin equivalents per gram extract.

Determination of antioxidant activity with the DPPH radical scavenging method:

Eighty milligrams of each extract were dissolved in 25 mL of methanol, sonicated for 20 min using ultrasonic bath (Clifton, England) and then 2 mL of this solution were added to 3 mL of DPPH solution (50 mg of DPPH/100 mL of methanol) and the volume was brought with methanol to 25 mL. The mixture was shaken vigorously and allowed to stand for 45 min in the dark at room temperature. The decrease in light absorbance was measured at 515 nm against a blank (without extract) using Spectronic 1201 spectrophotometer. The antioxidant activity was calculated using gallic acid as standard and the results were expressed as milligram gallic acid per gram extract (Matthaus, 2002).

Preparation of RBC: Human blood samples were collected into heparinized tubes and the Red Blood Cells (RBCs) were separated from plasma by centrifugation at 3000 rpm for 20 min. The crude RBCs were washed with the same volume of Phosphate Buffered Saline (PBS) pH 7.4 followed by centrifugation twice. The packed RBCs were then suspended in four volumes of PBS solution (Chen *et al.*, 1996).

Assay for free radical-mediated hemolysis: The method described by Miki *et al.* (1987) with slight modification was used. Two milliliters of RBC suspended in PBS (15%) was added to the same volume of 0.001 M FeSO₄ in PBS solution containing 100 µL of seed's extract. The reaction mixture was shaken gently in a rotary shaker at 37°C for 150 min. After incubation, 8 mL of PBS solution was added into the reaction mixture.

The diluted reaction mixture was then centrifuged at 3000 rpm for 10 min. The absorbance (A) of the supernatant at 450 nm was recorded in a Spectronic 1201 spectrophotometer. Percentage inhibition was calculated by the equation $(1 - A_{\text{antioxidant}} / A_{\text{FeSO}_4}) \times 100$, where A_{FeSO_4} is the absorbance of sample containing no extract and $A_{\text{antioxidant}}$ is the absorbance of sample containing extract.

Linoleic acid peroxidation assay (TBA method): The reaction mixture contained 500 µL of linoleic acid solution (20 mM), 500 µL Tris-HCl (100 mM, pH 7.5), 100 µL FeSO₄.7H₂O (4 mM) and 100 µL of plant seed extract. Linoleic acid peroxidation was initiated by the addition of 100 µL of ascorbic acid (2 mM). The reaction was incubated for 30 min at 37°C and terminated by the addition of trichloroacetic acid (5.5%). One milliliter of the mixture was added to 250 µL of thiobarbituric acid (TBA) in 50 mM NaOH, followed by heating for 10 min. The mixtures were centrifuged at 3500 rpm for 10 min and the

absorbance of the supernatant was read at 532 nm with Spectronic 1201 Spectrophotometer and converted into the percentage antioxidant activity using the following equation (Choi *et al.*, 2002):

$$\text{Linoleic acid peroxidation inhibition (\%)} = (\text{Ac}-\text{As}) \times 100 / (\text{Ac}-\text{An})$$

Where:

- Ac = Absorbance of control (with extract)
- As = Absorbance of extract
- An = Absorbance of blank (without extract and FeSO₄.7H₂O)

Determination of the reducing power: The reducing power of the extracts was determined by the method of Oyaizu (1988). Plant seed extract (2.5 mL) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%) and the mixture was incubated at 50°C for 20 min. Five milliliter of trichloroacetic acid (10%) were added to the reaction mixture, which was then centrifuged at 3000 rpm for 10 min. A 5 mL aliquot of the supernatant was mixed with 5 mL of distilled water and 1 mL of 1% ferric chloride and the absorbance was measured at 700 nm. An increase in the absorbance was taken as a measure of the reducing power of extract.

Statistical analysis: Data represent the mean of minimum of two replicate samples for each plant seed extract. Analysis of variance was performed using one-sample t-test and the significant differences (p<0.05) between the means were also determined.

RESULTS AND DISCUSSION

Results showed that all the methanolic plant seeds extracts possessed significant amounts of the phenolic content. More specifically, the extracts of *C. indica* exhibited the most total phenolic content among others. The phenolic content of the seven samples decreased in the following order: *C. indica* > *C. annuum* > *H. sativum* > *C. vulgaris* > *C. sinensis* > *T. sativum* (Table 1).

Table 1: Total phenolic and flavonoid contents of different plant seeds extracts

Plant seed extract	Polyphenols ------(mg g ⁻¹ DW)-----	Flavonoids
<i>C. sinensis</i>	2.330±0.416	1.480±0.076
<i>H. sativum</i>	3.670±0.223	2.560±0.132
<i>T. sativum</i>	0.406±0.005	0.060±0.010
<i>C. indica</i>	11.680±0.237	4.760±0.005
<i>C. vulgaris</i>	3.490±0.139	0.217±0.015
<i>C. annuum</i>	7.030±0.321	0.151±0.008

Values are means of three replicate analysis±SD

The higher amounts were found in the extracts of *C. indica* and *H. sativum* (4.76 and 2.55 mg g⁻¹ DW, respectively), followed by *C. sinensis* (1.48 mg g⁻¹ DW), *C. vulgaris* (0.22 mg g⁻¹ DW), *C. annuum* (0.15 mg g⁻¹ DW) and *T. sativum* (0.06 mg g⁻¹ DW). The flavonoids possess multiple health-promoting properties and have been studied in recent years for their structure and antioxidant activity (Das and Dereira, 1990; Gu and Weng, 2001).

The ability of the extracts to act as a free radical scavenger or hydrogen donor was revealed by DPPH, reducing power, inhibition of linoleic acid oxidation and inhibition of RBCs hemolysis methods for their radical scavenging activities.

The DPPH test is a method used for screening antioxidant molecules (Sanchez-Moreno *et al.*, 1998; Soler-Rivas *et al.*, 2000). In this method, the antioxidant will scavenge the DPPH radicals to form stable reduced DPPH molecules. The antioxidant radicals formed are stabilized through the formation of non-radical products (Argolo *et al.*, 2004).

The radical scavenging activity, using a DPPH generated radical, was tested with different sample extracts. It was observed (Fig. 1) that the extract of *C. indica* exhibited the highest radical- scavenging activity (87.12%), followed by extracts of *C. sinensis*, *C. vulgaris*, *T. sativum*, *C. annuum*. However, *H. sativum* showed the weakest activity (62.0%). These results reveal that *C. indica* and *C. sinensis* extracts contain powerful inhibitor compounds, which may act as primary antioxidants that react with free radicals. The higher the inhibitory activity is more potent the antioxidant

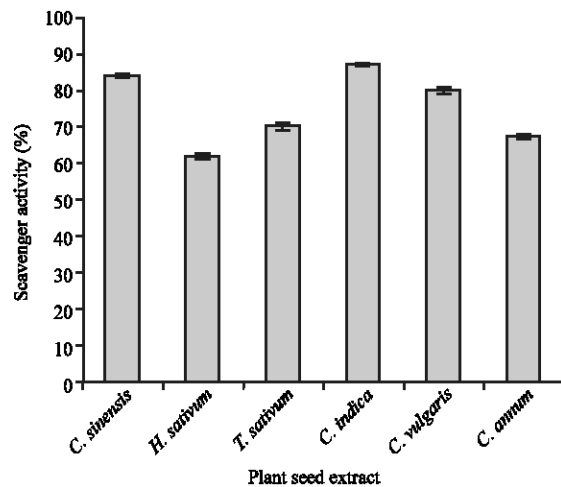


Fig. 1: Scavenging effects of different plant seeds extracts on DPPH radical. Values are means of three replicate analysis±SD

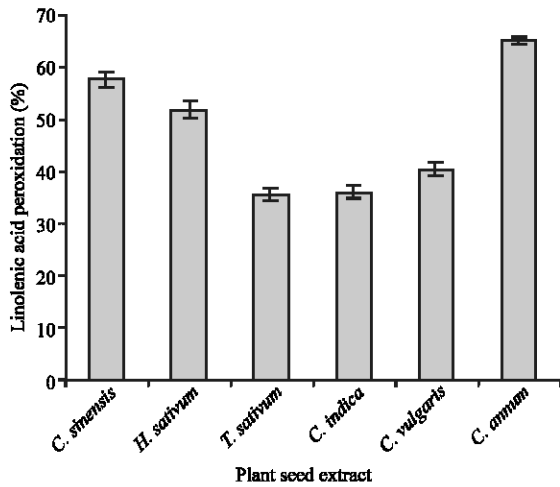


Fig. 2: Inhibition percentage (%) of linoleic acid oxidation by different plant seeds extracts in linoleate emulsion system. Values are means of two replicate analysis±SD

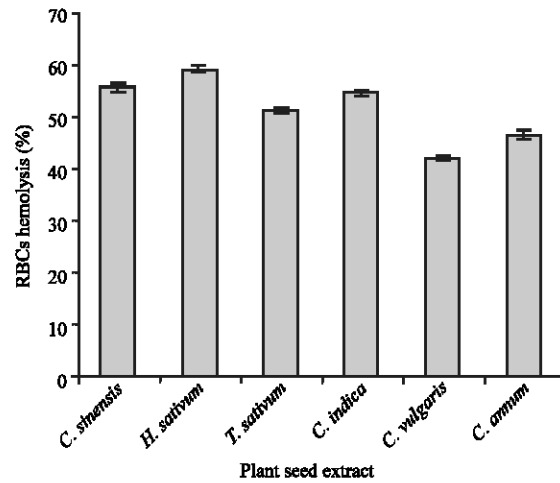


Fig. 3: Inhibitory effects (%) of different plant seeds extracts on free radical-induced lysis of human red blood cells. Values are means of two replicate analysis±SD

activity (Von Gadow *et al.*, 1997; Siddhuraju *et al.*, 2002). The elevated DPPH radical scavenging ability of the *C. indica* might be due to the presence of high contents of polyphenols (11.68 mg g⁻¹ DW) and flavonoids (4.76 mg g⁻¹ DW) (Table 1).

It was found that these extracts not only a DPPH radical- scavenging but also protect lipid molecules oxidation in the linoleic acid peroxidation method (Fig. 2) and inhibit lipid oxidation in the RBC membrane hemolysis method (Fig. 3). Lipid oxidation of human RBCs membrane mediated by AAPH induced initially membrane damage and subsequently hemolysis. *Hordeum sativum* was the most effective against lipid oxidation in the RBCs membrane followed by *C. sinensis*, *C. indica*, *T. sativum*, *C. annuum* and *C. vulgaris*.

The antioxidant effectiveness of the tested extracts in the RBCs suspension was generally similar to that in the linoleic acid peroxidation test, although the two systems were not comparable. In the linoleic acid experiment, the extract of *C. annuum* was the most potent inhibitory effect (65.06%) while *T. sativum* has the least effect (35.6%).

The antioxidative activity of the extracts tested is governed by their total number, location of hydroxyl groups, hydrophobicity/hydrophilicity and their interaction with other components in the RBC (Chen *et al.*, 1996).

It has been cited in the reports (Yen and Duh, 1993; Yen *et al.*, 2000) that the reducing power was associated with the antioxidant activity of polyphenolic compounds. In present study, we noticed that the reducing power was in the following order: *C. indica* > *H. sativum* > *C. annuum*

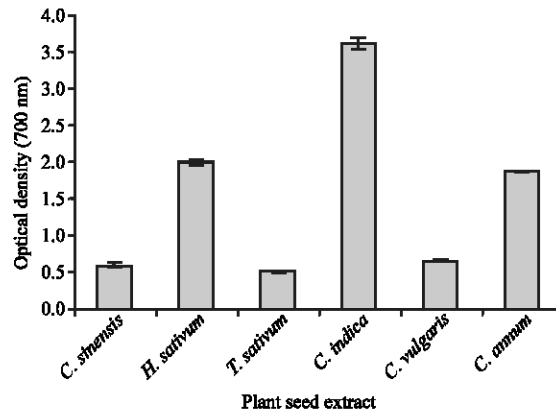


Fig. 4: Reducing power of different plant seeds extracts. Values are means of two replicate analysis±SD

> *C. vulgaris* > *C. sinensis* > *T. sativum*. Among all of the extracts, the *C. indica* showed a higher reducing power (3.61) and *T. sativum* exhibited the least (0.486) (Fig. 4).

In summary, our observations demonstrated that these extracts have different antioxidant activities. The antioxidant characteristics were determined by multiple factors including the system used, their hydrophobicity/hydrophilicity, the total number and location of hydroxyl groups on aromatic ring. It appears that the extract of *C. indica* is an effective antioxidant as a reducing power and DPPH inhibition and the extract of *H. sativum* is an effective protector against RBCs hemolysis, while the extract of *C. annuum* is effective against linoleic acid peroxidation.

CONCLUSION

The study clearly indicates that, it is important to measure the antioxidant activity using various radicals and oxidation systems and to take both phenolic content and antioxidant activity into account while evaluating the antioxidant potential of plant seeds extracts. However, the plant seeds extracts of *C. sinensis*, *H. sativum*, *T. sativum*, *C. indica*, *C. vulgaris* and *C. annuum* were used for determination of their phenolic and flavonoids contents and for their antioxidant activity by various antioxidant assays. Results showed that these methanolic extracts could be used as antioxidant in food and medicinal preparations, but further experiments and researches are required to confirm the pharmacological and toxicological studies.

ACKNOWLEDGMENTS

The author acknowledge the assistance of Dr. Saleh Quran (Department of Biological, Mutah University) for plant classification, Mr. Mohammad Garaibeh (Department of Mathematical, Mutah University) for statistical analysis and Yaseen Al-Qaisi (Department of Biological, Mutah University) for his help in the experiments.

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