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IN VITRO ANTIOXIDANT ACTIVITY OF SELECTED EGYPTIAN PLANTS

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ABSTRACT

Free radicals react with biological molecules and destroy the structure of cells, which eventually causes free radical induced diseases such as cancer, liver disease, aging, etc.

In this study, the total phenolic contents, 1, 1- diphenyl-2-picryl hydrazyl ($DPPH^{\cdot}$) and $OH^{\cdot}$ radical scavenging activity and lipid peroxidation of 13 selected Egyptian plants were determined. Total phenolic contents of plant ethanolic extracts were measured as gallic acid. The antioxidant activities of plant ethanolic extracts at different levels (25, 50 and 100 µg/ ml) were evaluated using three complementary *in vitro* assays: inhibition of $DPPH^{\cdot}$ radical, hydroxyl radical and lipid peroxidation in liver homogenate mediated by $FeSO_4$/ ascorbate model system. The results showed that all plant ethanolic extracts increased antioxidant activities with increasing ethanolic extracts concentration. The ethanolic extracts of guava leaves, cinnamon bark and pomegranate peel significantly inhibited lipid peroxidation- induced by $FeSO_4$/ ascorbate model system, also have inhibitory effect on deoxyribose degradation. In addition, guava leaves had the highest free radical scavenging activity. These findings suggest that ethanolic extracts of guava leaves, cinnamon bark and pomegranate peel are powerful natural antioxidants and may be useful as antioxidants interest in the protection of biological system against various oxidative stresses. The chemical constitution of ethanolic extracts was investigated using gas chromatography–mass spectrometry (GC–MS) for the guava leaves and pomegranate peel. The ethanolic extracts were found to contain 19 and 23 components for guava leaves and pomegranate peel, respectively. The major peaks, identified by GC–MS, were 1,2-Benzenedicarboxylic acid, diisooctyl ester (28.72%), 5- Methyl-2-phenylindole (20.80%) and n-Octanoic acid (19.00%) for the guava leaves and 5-Hyroxymethyl- 2-formylfuran (68.35%), 2-Furancarboxaldehyde (6.94%) and 3,4-Dehydroproline (3.78%) for the pomegranate peel**.**

Keywords: Plant ethanolic extracts; phenolic content; antioxidant activity; 1,1-diphenyl 2-picryl hydrazyl (DPPH); hydroxyl radical; lipid peroxidation; GC–MS.

INTRODUCTION

Humans is continuously exposed to different kinds of chemicals such as food additives, industrial chemicals, pesticides and other undesirable contaminants in the air, food and soil (Stavric, 1994). Most of these chemicals induce a free radical-mediated lipid peroxidation leading to disruption of biomembranes and dysfunction of cell and tissues (Cho *et al.,* 2003). Antioxidants play a significant role in protecting living organism from the toxic effect of various chemicals by preventing free radical formation (Sheweita *et al.*, 2001). Potential sources of antioxidant compounds have been searched

in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks, roots, spices, herbs, and crude plant drugs (Ramarathnam *et al*., 1995). Recent studies showed that the phytochemicals; especially phenolics; in fruits and vegetables are the major bioactive compounds with human health benefits (Wang *et al*., 1996). There are approximately 5000 known plant phenolics and model studies have demonstrated that many of them have antioxidant activity (Robards *et al*., 1999). In the case of phenolic compounds, the ability of the phenolics to act as antioxidants depends on the redox properties of their phenolic hydroxyl groups that allow them to act as reducing agents, hydrogen-donating antioxidants and singlet oxygen quenchers (Rice-Evans *et al*., 1995). Their antioxidant activity is generally based on the number and location of hydroxyl groups present as well as the presence of a 2-3 double bond and 4-oxofunction (Rice-Evans and Miller, 1998).

Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Natural antioxidant substances are presumed to be safe since they occur in plant foods, and are seen as more desirable than their synthetic counterparts. Data from both scientific reports and laboratory studies show that plants contain a large variety of substances called ‘‘plant chemicals’’ or ‘‘phytochemicals’’ that possess antioxidant activity (Pratt, 1992). Natural antioxidants occur in all higher plants, and in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen, and seeds). Typical compounds that exhibit antioxidant activity include vitamins, carotenoids, and phenolic compounds. Therefore, recommendations have been made to increase the daily intake of fruit and vegetables, which are rich in these nutrients that lower the risk of chronic health problems associated with the diseases mentioned above (Slattery *et al.,* 2000). The antioxidant compounds of higher plants have been demonstrated, *in vitro* experiments, to protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species. The roles of these compounds as potential antioxidants can be inferred by their similarity to synthetic antioxidants, of related structures (Larson, 1988). The growing interest in the antioxidant properties of the phenolic compounds in vegetables and fruits derives from their strong activity and low toxicity compared with those of synthetic phenolic antioxidants, such as BHT (butylated hydroxytoluene) (Marinova and Yanishlieva, 1997).

More attention has been paid to the role of natural antioxidants mainly phenolic compounds, which may have more antioxidant activity than vitamins C and E, β-carotene (Vinson *et al.,* 1995), and lycopene (a carotenoid without provitamin A) (Giovannucci *et al.,* 2002). The antioxidative effects of natural phenolic compounds in pure forms or in their extracts from different plant sources such as vegetables, fruits and medicinal plants were studied *in vitro* using different model systems of oxidation (Pietta *et al.,* 1998; Yen and Hsieh, 1998) led to speculation about the potential benefits of ingestion of plant phenolics. Therefore, antioxidants, which can neutralize free radicals, may be of central importance in the prevention of carcinogenicity, cardiovascular and neurodegenerative changes associated with aging (Halliwell, 1994). Epidemiological studies show that the

consumption of vegetables and fruits can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (Ames *et al.*, 1993).

The aim of this study was to screen a number of plant material that are widely used in Egypt to evaluate their total phenolic content and *in vitro* antioxidant activities in order to find new potential sources of natural antioxidants. The antioxidant activities were determined by three *in vitro* assays: inhibition of $DPPH^{\cdot}$ radical, hydroxyl radicals and lipid peroxidation in liver homogenate mediated by $FeSO_4$/ ascorbate model system. Another aim was to identify compounds which are responsible for these activities by GC–MS.

MATERIALS AND METHODS

Chemicals

All reagents were of analytical grade. Chemicals were purchased from Sigma Chemicals Co. (St Louis, MO, USA) and Solvents were purchased from Merck (Darmstadt, Germany).

Plant material

All plants used in this experiment were purchased from local markets in Cairo, Egypt, during June–October 2006. The scientific names of the plants which were used in the study are given in Table (1).

Table (1): Edible plants used in this study

Arabic name	English name	Scientific name	Family name	Part used
الرمان	Pomegranate	*Punica granatum*	Punicaceae	Peel & Seed
القرفة	Cinnamon	*Cinnamomum zeylanicum*	Lauraceae	Bark
الجوافة	Guava	*Psidium guajava*	Myrtaceae	Leaves
الكركدية	Roselle	*Hibiscus sabdariffa* L.	Malvaceae	Flowers
العنب الأحمر	Red grape	*Vitis vinifera* cv. Flam seed less	Vitaceae	Fruits & Leaves
الفراولة	Strawberry	*Fragaria ananassa* Duch.	Rosaceae	Fruits
الفجل	Radish	*Raphanus sativus* cv. White lcicle	Brasicaceae	Leaves
التوت الأسود	Black mulberry	*Morus nigra* L.	Moraceae	Fruits
البرقوق الأحمر	Plums	*Prunus salicina* Var. *Hheluode*	Rosaceae	Fruits
التفاح	Apple	*Pyras mallus* Var *Anna*	Rosaceae	Fruits
التين البرشومى	Fig	*Ficus* carica L.	Moraceae	Fruits
الخوخ	Peaches	*Prunus persica* Var. *Earlygrand*	Rosaceae	Fruits
الكرنب	White cabbage	*Brassica oleracea* Var *Capitata* L.	Brasicaceae	Leaves

Preparation of plant extracts

Plants were flushed by tap water then washed in distilled water three times and cut into small pieces before being dried in a hot air-blowing oven at 50°C. They were ground to a fine powder in a mechanical blender. 10 g of each dry powder were extracted with 100 ml of 70% ethanol in a screw-capped flask and shaken at room temperature for 24 h. The extracts were centrifuged at 5000*g* for 10 min while the residue was re-extracted under the same conditions twice and filtered through a Büchner funnel with filter paper

(Whatman No.1). The ethanol extracts were concentrated under reduced pressure, lyophilized to obtain powders, and stored at 4 °C until assay.

Antioxidant activity toward lipid peroxidation in rat liver mitochondria. Preparation of rat liver mitochondria.

Male albino rats weight about 200±5g were obtained from Faculty of Veterinary Medicine, Cairo University. The rats were raised in the animal's house of Regional Center for Food and Feed, Agriculture Research Center. Food and water were provided *ad libitum*. The animals were fasted overnight and the day after were killed and the liver from each animal was collected. Mitochondria were isolated from liver tissue by the method of Kimura *et al.* (1984). The liver tissue were cut into small slices in 0.25 M sucrose containing 3 mM Tris-HCl and 0.1 mM EDATA (pH 7.4) at 4°C and then small slices of liver were Homogenized with 9-fold 0.25 M sucrose solution containing 3 mM Tris-HCl and 0.1 mM EDATA (pH 7.4) at 4°C using a teflon homogenizer. The homogenate solution was adjusted to pH 7.4 by addition of 0.1 N KCl and then the homogenate was centrifuged at 50 xg for 10 min at 4°C to remove the nuclear fractions and blood red cells. The supernatant phase was centrifuged at 700xg for 10 min at 4°C and then the supernatant was further centrifuged at 5000xg for 45 min at 4°C to give mitochondria fractions. The isolated mitochondria fractions were twice washed with Krebs-Ringer phosphate buffer (0.122 M NaCl, 1.023 mM MgSO4.7H2O, 4.87 mM KCl and 41.7 mM NaHPO4.12H2O) (pH 7.4).

Lipid peroxidation assay.

The effect of different types of plant ethanolic extracts on $FeSO_4$/ascorbate-induced peroxidation in rat liver mitochondria was determined by the method of Afanas'ev *et al.* (1989). Reaction mixture contained 200 µl $FeSO_4$ (0.075 M), 500 µl of mitochondria suspension, 100 µl of plant ethanolic extracts or rutin as standard were dissolved in MeOH in different concentrations (25, 50 and 100 µg/ ml), 200 µl of L-ascorbic acid (0.1M) and phosphate buffer (pH 7.4, 0.1 M) up to a final volume of 4 ml. Samples were incubated 1 h at 37 °C. Then, 200 µl of EDTA (0.1 M) and 1.5 ml of TBA reagent (3 g TBA, 120 g TCA and 10.4 ml $HClO_4$ in 800 ml of distilled water) were added to each sample and heated for 15 min at 100 °C. After cooling on ice, samples were centrifugated for 10 min (3000 rpm) and the color reaction of the MDA–TBA complex in supernatant was measured at 532 nm using a spectrophotometer.

Evaluation of the hydroxyl radical scavenging activity.

Hydroxyl radical scavenging activity was carried out by measuring the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system as described by Halliwell *et al.* (1987). Attack of the hydroxyl radical on deoxyribose led to TBARS (thiobarbituric acid-reactive substances) formation. 500 µl of plant ethanolic extracts or rutin as standard in different concentrations (25, 50 and 100 µg/ ml) were dissolved in phosphate buffer (20 mM, pH 7.4) and added to the reaction mixture containing (100 µl 2.8 deoxyribose mM, 100 µl $FeCl_3$100 µM, 100 µl EDTA 104 µM, 100 µl ascorbic acid 100 µM and 100 µl H_2O_2 1 mM). The mixtures were incubated 1 h at 37 °C, and then 1 ml of 1%

(w/v) thiobarbituric acid (TBA) in 0.05 M NaOH and 1 ml of 2.8% (w/v) trichloroacetic acid (TCA) were added in each mixture and heated 15 min at 100 °C. After cooling on ice, absorbance was measured at 532 nm using a spectrophotometer.

Free radical scavenging activity in 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$) assay.

The antioxidant activity of plant ethanolic extracts was measured using a Free radical scavenging activity method of Blois (1958) and Brand-Williams *et al.* (1995). The free radical used in this study was 1,1-Diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$). Different concentrations (25, 50 and 100 µg/ ml) of plant ethanolic extracts and rutin as standard were taken in different test tubes. The volume was adjusted to 100µl by adding methanol. 3.9 ml of 6×10^{-5} mol/L DPPH solution made up with DPPH (4.8 mg) in methanol (200 mL) were added to these tubes. The mixture was shaken and left to stand at room temperature for 1 h. Absorbance of the resulting solution was measured at 517 nm by a UV–Visible spectrophotometer. The readings were compared with the controls, which contained 100 µl of methanol instead of the extract.

Determination of total phenolics contents

The concentration of phenolic compounds in plant ethanolic extracts were determined by Folin–Ciocalteu method (Folin and Ciocalteu, 1927).

Identification of plant ethanolic extracts compounds using gas chromatography-mass spectrometry (GC–MS).

The plant ethanolic extracts were analyzed according to Harvey (1981) using a Hewelett Packard gas chromatography- mass spectrometry model 6890 series equipped with selective detector mass spectroscopy model 5973. This equipment was interfaced via HP chemstation version A02.12 software (Hewelett-Packard, Avondale, P.A.). The gas chromatography was equipped with capillary column HP-5.MS HP length 80 cm and thickness 0.3 mm. The operating conditions for gas chromatography were as follows: the injection temperature was 290 ºC, the carrier gas (Helium) flow rate was 0.8 ml/min, the oven temperature program was from 100 ºC (5 min) raised at 150-300 ºC (10 min) and final temperature for 15 min, the detector temperature was 320 ºC. Two microliters of the sample was injected. Mass spectroscopy was operated first in scanning model in mass range from 40 to 540 m/z and identification was based on standard mass library (NIST version 2.0). The separated components were identified by matching them with the National institute of Standards and Technology (NIST) mass spectral library data. The quantitative determination was carried out by peak area integration.

Statistical Analysis

One way analysis of variance was used to compare the data, and the values were considered statistically significant differences in the Duncan test at $P < 0.05$.

RESULTS

The percentage inhibition of free radical formation in different systems assay, e.g. DPPH assay, hydroxyl radical-scavenging assay and lipid peroxidation assay and total phenolic content of plant ethanolic extracts were determined spectrophotometrically.

Inhibition of Mitochondrial Lipid Peroxidation

Redox reactions frequently occur in mitochondria, which are constantly susceptible to oxidative stress. In particular, the inner membranes of mitochondria are at risk from lipid peroxidation, because mitochondria utilize oxygen at a high rate and inner membranes have a large content of polyunsaturated fatty acids, together with peroxidation catalysts such as iron and copper (Hingh *et al.,* 1995). As shown in Table (2), rutin, a reference substance, exhibited a concentration-dependent suppressive effect on the lipid peroxidation caused by the radical-generating system in rat liver mitochondria. Guava leaves**,** cinnamon bark and pomegranate peel were significantly better inhibitors for the formation of TBARS in mitochondria suspension in a concentration-dependent manner than the others, compared to rutin. No significant difference was found between the high concentration of rutin and guava leaves in their inhibiting lipid peroxidation.

Table (2): Inhibitory effect of different plant ethanolic extracts and rutin against lipid peroxidation in rat liver mitochondria.

Plant ethanolic extracts	Part used	*% Inhibition of lipid peroxidation		
		25 µg/ml	50 µg/ml	100 µg/ml
Rutin	Standard	89.17±0.519^{a}	95.35±0.259^{a}	98.80±0.449^{a}
Pomegranate	Peel	73.35±0.766^{d}	80.27±1.482^{d}	90.92±0.519^{c}
Cinnamon	Bark	80.38±0.490^{c}	91.04±0.519^{c}	95.57±0.449^{b}
Guava	Leaves	87.52±0.967^{b}	93.76±0.766^{b}	98.29±0.340^{a}
Roselle	Flowers	30.32±0.427^{f}	36.56±1.283^{f}	51.41±0.936^{e}
Grape	Leaves	35.82±1.374^{e}	56.46±1.020^{e}	74.20±0.597^{d}
Strawberry	Fruits	6.80±0.340^{k}	11.39±0.946^{jk}	13.20±0.259^{ij}
Pomegranate	seeds	5.49±0.936^{l}	10.09±0.996^{k}	14.17±0.936^{hi}
Radish	Leaves	3.00±0.354^{m}	4.70±1.132^{m}	10.20±1.558^{k}
Black Mulberry	Fruits	10.60±0.936^{i}	16.78±0.519^{i}	18.02±1.020^{g}
Plums	Fruits	21.99±1.194^{g}	31.51±0.643^{h}	47.84±0.687^{f}
Apple	Fruits	18.99±0.855^{h}	33.04±0.597^{g}	48.80±0.779^{f}
Fig	Fruits	8.33±0.741^{j}	12.30±0.936^{j}	15.41±0.392^{h}
Peaches	Fruits	2.77±0.546^{m}	7.65±0.613^{l}	11.90±1.283^{j}
Red Grape	Fruits	0.73±0.098^{n}	2.94±0.597^{n}	7.31±0.779^{l}
Cabbage	Leaves	2.49±0.259^{m}	3.68±0.427^{mn}	7.59±0.427^{l}
		LSD 0.05= 1.259	**LSD0.05= 1.429**	**LSD 0.05= 1.321**

*** Inhibition (%) was expressed as the absorbance of sample with the absorbance of control**

Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

The various superscript letters indicate statistically significant differences in the Duncan test, with *P* <0.05.

Hydroxyl Radical-Scavenging Activity.

The deoxyribose method is a simple assay to determine the rate constants for reactions of hydroxyl radicals **(Halliwell *et al*. 1987)**. When the mixture of $FeCl_3$-EDTA, H_2O_2, and ascorbate was incubated with deoxyribose in phosphate buffer (pH 7.4), the hydroxyl radicals generated attack the deoxyribose, resulting in a series of reactions that caused the formation of malonaldehyde (MDA). Any hydroxyl radical scavenger added to the reaction would compete with deoxyribose for the availability of hydroxyl radicals, thus reducing the amount of MDA formation. As shown in Table (3), it is clear that the inhibition effect of extracts against deoxyribose degradation was dose-dependent manner. Guava leaves, cinnamon bark and pomegranate peel extracts gave the highest preventing value against deoxyribose degradation induced by hydroxyl radicals. Complete inhibition of the deoxyribose degradation was at 100 µg/ ml of guava leaves extract as well as rutin.

Table (3): Inhibitory effect of different plant ethanolic extracts and rutin against hydroxyl radical mediated deoxyribose degradation.

Plant ethanolic extracts	Part used	*% Inhibition of deoxyribose degradation		
		25 µg/ml	50 µg/ml	100 µg/ml
Rutin	Standard	86.21±0.690^{a}	98.27±0.19^{a}	100±0.0^{a}
Pomegranate	Peel	34.84±0.773^{d}	57.81±0.552^{d}	83.53±0.877^{c}
Cinnamon	Bark	44.41±0.584^{c}	76.96±0.481^{c}	86.72±0.672^{b}
Guava	Leaves	62.41±0.797^{b}	92.27±0.584^{b}	100±0.0^{a}
Roselle	Flowers	12.06±0.481^{f}	29.48±0.398^{f}	69.04±0.773^{e}
Grape	Leaves	14.61±0.690^{e}	33.12±0.773^{e}	75.17±0.292^{d}
Strawberry	Fruits	6.89±1.344^{ghi}	12.82±0.904^{i}	24.56±0.506^{i}
Pomegranate	seeds	6.31±0.773^{hij}	12.12±0.834^{ij}	23.35±1.153^{ij}
Radish	Leaves	5.87±0.982^{ijk}	11.55±0.574^{j}	22.39±1.054^{j}
Black Mulberry	Fruits	7.59±0.904^{gh}	16.46±0.863^{h}	36.75±0.584^{h}
Plums	Fruits	11.48±1.121^{f}	28.97±0.765^{f}	66.11±1.065^{f}
Apple	Fruits	8.04±0.724^{g}	19.97±0.574^{g}	45.18±0.773^{g}
Fig	Fruits	5.61±0.877^{ijk}	8.93±0.481^{k}	17.61±0.584^{k}
Peaches	Fruits	4.97±0.863^{jkl}	8.23±0.944^{kl}	10.72±1.054^{l}
Red Grape	Fruits	4.08±0.506^{l}	5.74±0.672^{m}	7.91±0.877^{m}
Cabbage	Leaves	4.65±0.834^{kl}	7.65±0.398^{l}	8.80±0.797^{m}
		LSD 0.05= 1.391	**LSD0.05= 1.093**	**LSD 0.05= 1.286**

*** Inhibition (%) was expressed as the absorbance of sample with the absorbance of control**

Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

The various superscript letters indicate statistically significant differences in the Duncan test, with *P* <0.05.

DPPH Radical-Scavenging Activity.

DPPH• is a stable free radical. Antioxidants, on interaction with DPPH•, either transfer electrons or hydrogen atoms to DPPH•, thus neutralising free radical character (Naik *et al.* 2003). The colour of the reaction mixture changes from purple to yellow and its absorbance at wavelength 517 nm decreases. The scavenging effect of 15 selected common plant ethanolic extracts on DPPH radical changed significantly (P <

0.05) at the different concentrations are shown in Table (4). Guava leaves, cinnamon bark, and pomegranate peel had the highest antioxidant activities, respectively. Singh *et al.* (2002) recently reported that methanol extract of pomegranate peel had much higher antioxidant capacity than that of seeds, as demonstrated by using the DPPH model systems. Antiradical activity increased significantly with the increasing extract concentrations for all samples. Antioxidant activity of guava leaves extract at low concentration was similar to that of rutin (a control antioxidant). In particular, research has focused on a search for antioxidants of extracts from leaves of various guava cultivars. The extracts from guava leaves exhibited more scavenging effects on free radicals than did commercial guava tea extracts and dried fruit extracts (Chen and Yen, 2007).

Table (4): Free Radical scavanging actvity of different plant ethanolic extracts and rutin by DPPH• assay.

Plant ethanolic extracts	Part used	*% Inhibition of DPPH•		
		25 µg/ml	50 µg/ml	100 µg/ml
Rutin	Standard	91.75±0.717^{a}	96.21±0.126^{a}	96.84±0.252^{a}
Pomegranate	Peel	43.03±0.525^{c}	78.24±0.405^{d}	95.16±0.072^{c}
Cinnamon	Bark	62.09±1.199^{b}	91.79±0.455^{c}	95.79±0.072^{bc}
Guava	Leaves	91.08±0.317^{a}	95.37±0.262^{b}	95.96±0.126^{b}
Roselle	Flowers	6.56±0.317^{h}	9.54±0.364^{h}	23.72±1.058^{e}
Grape	Leaves	15.69±0.952^{d}	55.53±1.264^{e}	64.03±0.788^{d}
Strawberry	Fruits	2.56±0.333^{i}	4.83±0.455^{k}	10.47±0.364^{h}
Pomegranate	seeds	2.27±0.364^{ij}	4.62±0.145^{k}	10.30±0.405^{h}
Radish	Leaves	2.77±0.930^{j}	7.61±0.550^{j}	9.84±0.443^{h}
Black Mulberry	Fruits	10.64±0.378^{f}	11.27±0.378^{g}	12.57±0.385^{g}
Plums	Fruits	12.87±0.072^{e}	13.04±0.126^{f}	15.35±0.145^{f}
Apple	Fruits	10.26±0.437^{f}	11.40±0.455^{g}	15.06±0.378^{f}
Fig	Fruits	7.90±0.145^{g}	8.20±0.192^{i}	8.66± 0.443^{i}
Peaches	Fruits	5.76±0.192^{h}	6.01±0.072^{j}	7.36±0.126^{j}
Red Grape	Fruits	1.34±0.072^{j}	2.60± 0.145^{l}	3.87±0.262^{l}
Cabbage	Leaves	2.01±0.192^{ij}	4.83± 0.378^{k}	6.05± 0.385^{k}
		LSD 0.05= 0.919	**LSD0.05= 0.754**	**LSD 0.05= 0.726**

*** Inhibition (%) was expressed as the absorbance of sample with the absorbance of control**

Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

The various superscript letters indicate statistically significant differences in the Duncan test, with *P* <0.05.

Total Phenolic Content

Phenolic compounds, or polyphenols, constitute one of the most numerous and widely distributed groups of substances in plant kingdom. They range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins. In general, antioxidant and radical scavenging properties of plant extracts are associated with the presence of phenolic compounds possessing the ability to donate hydrogen to the radical. The total amount of phenolic compounds in plant ethanol extracts are shown in Table (5). Significantly, the highest amounts were found in the order: rutin (as a reference compounds**) >** Pomegranate Peel > Cinnamon bark, Guava leaves > Roselle flowers, Grape leaves (P < 0.05). However, cabbage leaves

had the lowest content of total phenolic compounds. Phenolic substances have been shown to be responsible for the antiradical activity of plant materials (Rice-Evans *et al.,* 1996).

Table (5): Total phenolic content in the ethanolic extracts of different plants and rutin as gallic acid equivalents (GAE).

Plant ethanolic extracts	Part used	Total phenolic content (mg GAE/ 100 g ethanolic extracts)
Rutin	Standard	394.12± 8.53[a]
Pomegranate	Peel	385.31± 3.39[b]
Cinnamon	Bark	370.76±7.05[c]
Guava	Leaves	366.38± 2.91[cd]
Roselle	Flowers	361.21± 3.41[de]
Grape	Leaves	354.13± 2.38[e]
Strawberry	Fruits	90.02± 2.80[f]
Pomegranate	seeds	74.67± 4.70[g]
Radish	Leaves	74.67± 1.68[g]
Black Mulberry	Fruits	72.56± 0.15[gh]
Plums	Fruits	65.78± 5.87[h]
Apple	Fruits	34.09± 7.68[i]
Fig	Fruits	31.39± 0.45[i]
Peaches	Fruits	29.30± 5.47[i]
Red Grape	Fruits	23.38± 3.25[j]
Cabbage	Leaves	12.90± 6.74[k]
		LSD 0.05= 0.080

Each value in the table was obtained by calculating the average of three experiments ± standard deviation.
The various superscript letters indicate statistically significant differences in the Duncan test, with *P* <0.05.

GC–MS of the ethanolic extracts

Guava leaves

Fig. 1 shows the GC–MS chromatographic separation of compounds of the guava leaves ethanolic extract. A total of nineteen compounds were identified Table (6).The major compounds, which were identified by GC–MS, were 1,2-Benzenedicarboxylic acid, diisooctyl ester (28.72%), 5- Methyl-2-phenylindole (20.80%), n-Octanoic acid (19.00%), 5-Hyroxymethyl- 2-Furfural (11.72%), 1- Methyl-2-phenylindole (7.15%), 3,5-Dihyroxy-2- methyl-5,6-dihydropyran-4-one (3.16%), Pyrogallic acid (1.86%), 2-Methyl- 5-(methylthio) Furan (1.28%) and 4,4 dimethyl-5 alpha - cholan -3-one-24-oate (1.12%).

Pomegranate peel

Fig. 2 shows the GC–MS chromatographic separation of compounds of the pomegranate peel ethanolic extract. A total of twenty-three compounds were identified Table (7). The major compounds, which were identified by

GC–MS, were 5-Hyroxymethyl- 2-Furfural (68.35%), 2-Furancarboxaldehyde (6.94%), 3,4-Dehydroproline cyclohexanone (3.78%), 5,5´-Oxydimethylene-di-2-furaldehyde (3.11%),2-Furancarboxylic acid methyl ester (2.44%), Palmitic acid methyl ester (1.63%), 3-Methyl-2- Fumaric acid (1.37%) and 2,3-Dihydro-3,5-dihyroxy-6-methyl-4H-pyran-4-one (1.26%).

Table (6): Compounds identified in guava leaves ethanolic extracts by gas chromatography–mass spectrometry (GC–MS).

Peak no.	Rt.[a] (min)	Conc. %[b]	m/z	Compounds
1	3.164	0.86	96	2-Furancarboxaldehyde
2	3.414	0.69	98	2-Furanmethanol
3	4.973	0.61	114	4-Hydroxycyclohexanone
4	5.631	1.28	128	3-Fluoroctechol
5	6.315	3.16	144	2,3-Dihydro-3,5 dihyroxy-2- methyl- 4H-pyran-4-one
6	6.859	0.93	120	Coumaran
7	7.065	11.72	126	5-Hyroxymethyl- 2-formylfuran
8	8.473	1.86	126	Pyrogallic acid
9	8.798	0.15	182	2-Propenoic acid,3-(2,2,3,3-tetramethylcyclopropyl methyl ester
10	9.134	0.23	284	Stearic acid
11-12	9.241-9.458	0.60	340	1-Formyl-2,2,6-trimethyl-3-cis-(3- methyl but-2-enyl)-5-cyclohexane
13	9.536	0.19	414	Adpidospetmidin-17-ol,1-acyl-19,21-epoxy-15,16-dimethoxy
14	9.730	0.47	154	Citronellal
15-17	10.039-10.216	19.00	146	n-Octanoic acid
18	11.266	7.15	207	1- Methyl-2-phenylindole
19-20	11.527-11.594	28.72	279	1,2-Benzenedicarboxylic acid, diisooctyl ester
21-23	11.855-12.396	20.80	207	5- Methyl-2-phenylindole
24	12.695	0.48	282	(1S,2S)-2-((Dipheny) hyroxymethyl) cyclohexanol
25	12.885	1.12	416	4,4 dimethyl-5 alpha - cholan -3-one-24-oate, methyl ester

[a]Retention time (min)
[b]Conc. %: the percent of concentrations based on peak area integration

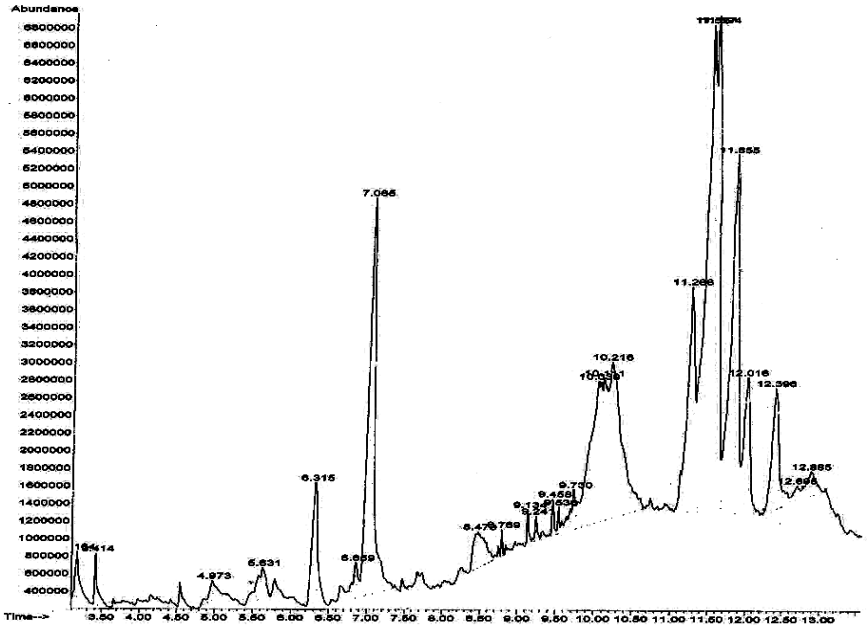
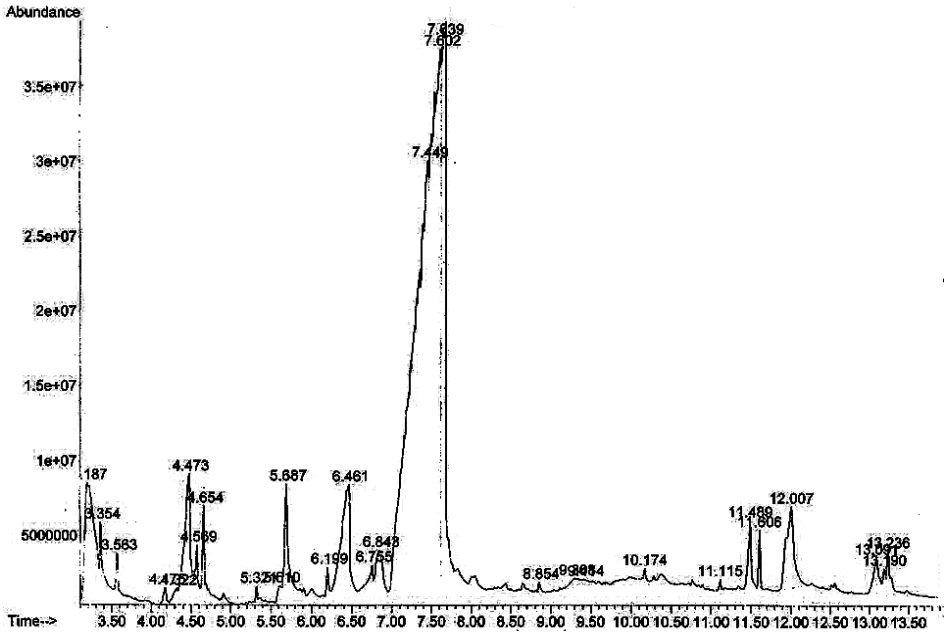


Fig. (1) GC- MS chromatogram of guava leaves ethanolic extract.

Table (7): Compounds identified in pomegranate peel ethanolic extracts by gas chromatography–mass spectrometry (GC–MS).

Peak no.	Rt.[a] (min)	Conc. %[b]	m/z	Compounds
1-2	3.187-3.354	6.94	96	2-Furancarboxaldehyde
3	3.563	0.88	98	2-Furanmethanol
4	4.173	0.25	84	2 (5H) -Furanone
5	4.323	0.33	111	6-methyl-2,5-Furandione
6	4.473	3.78	110	3,4-Dehydroproline
7	4.569	0.84	110	5-Methyl- 2-Furfural
8	4.654	1.26	144	2,3-Dihydro-3,5-dihyroxy-6-methyl-4H-pyran-4-one
9	5.321	0.08	126	4-Ethyl cyclohexanone
10	5.610	0.28	124	Orcinol
11	5.687	2.44	126	2-Furancarboxylic acid methyl ester
12	6.199	0.32	140	2,2- Dimethyl-1-oxa-2-silacyclo-3,5-hexadiene
13	6.463	4.71	144	5,6-Dihydro-3,5-dihyroxy-2-methyl-4H-pyran-4-one
14	6.755	0.75	144	Fumaric acid dimethyl ester
15	6.843	1.37	111	3-Methyl-2- Furoic acid
16-18	7.449-7.639	68.35	126	5-Hyroxymethyl- 2-formylfuran
19	8.854	0.11	109	2-Aminophenol
20-21	9.308-9.414	0.46	146	n-Octanoic acid
22	10.174	0.12	228	n-Tetradecanoic acid
23-25	11.115-11.606	1.63	256	Palmitic acid
26	12.007	3.11	234	5,5´-Oxydimethylene-di-2-furaldehyde
27	13.091	0.93	282	Oleic acid
28	13.190	0.31	308	Linoleic acid ethyl ester
29	13.236	0.74	310	Stearic acid ethyl ester

[a]**Retention time (min)**



[b]Conc. %: the percent of concentrations based on peak area integration.
Fig. (2) GC- MS chromatogram of pomegranate peel ethanolic extract.

DISCUSSION

Interest in the search for new natural antioxidants has grown dramatically over the past years, because reactive oxygen species production and oxidative stress has been shown to be linked to ageing related illnesses (Finkel and Holbrook, 2000) and a large number of other illnesses. So, the aim of this study was to evaluate antioxidant activity (*in vitro*) of a large number of plant materials that are widely consumed in Egypt.

We initially used a Fe^{3+}-dependent system to test the scavenging activity of these plant ethanol extracts on radicals generated by iron, because hydroxyl radicals are known to be the most reactive of all the reduced forms of dioxygen and are thought to initiate cell damage *in vivo* (Rollet-Labelle *et al.,* 1998). The results of lipid peroxidation assay showed that the ethanolic extracts of guava leaves, cinnamon bark and pomegranate peel were the most active scavenger of hydroxyl radicals. Next, we use deoxyribose assay system to confirm the antioxidant activity of ethanolic extracts of guava leaves, cinnamon bark and pomegranate peel. They showed strong inhibition effects on hydroxyl radicals induced deoxyribose degradation assay. The indirect evidence of the scavenging activity of plant extract on Fe^{3+}-dependent hydroxyl-radical generation was further confirmed using a direct approach with DPPH• radicals, a stable radical used to evaluate the antioxidant activity of plant extracts (Hu and Kitts, 2000 and Chang *et al.,* 2001). In this assay,

the ethanol extracts of guava leaves, cinnamon bark and pomegranate peel exhibited powerful DPPH radical scavenging activity and the activity were similar to that of rutin in the concentrstion100 µg/ml of these plant extracts, suggesting that guava leaves, cinnamon bark and pomegranate peel extracts are powerful natural antioxidant.

Finally, we examined the phenolic compounds content of the plant ethanol extracts using the Folin-Ciocalteu assay, because phenolic compounds are commonly found in plants and are reported to have multiple biological effects, including antioxidant activity (Miranda *et al.,* 1999). In similar studies, the enrichment of phenolic compounds within plant extracts is correlated with their enhanced antioxidant activity (Yen and Hsieh 1998 and Lee *et al.* 2002). This clearly explained the reason for the antioxidant activity of guava leaves, cinnamon bark and pomegranate peel.

In summary, it is well understood that the generation of reactive oxygen species beyond the capacity of a biological system to eliminate them gives rise to oxidative stress. This stress may play a role in several diseases, such as heart disease, degenerative neuronal disease and cancer (van Poppel and van den Berg, 1997; Mates and Sanchez-Jimenez, 2000; Adams and Odunze, 1991 and Hertog *et al*., 1993). Furthermore, many biochemical and clinical studies suggest that natural and synthetic antioxidant compounds are helpful in treating disease mediated by oxidative stress. This study demonstrated that the ethanolic extracts of guava leaves**,** cinnamon bark and pomegranate peel have excellent antioxidant activities. In further experiments, it would be interesting to investigate the antioxidant potential *in vivo* of the ethanolic extracts from guava leaves and pomegranate peel.

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تقدير النشاط المضاد للأكسدة معمليا لبعض النباتات المصرية المختارة

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الشقوق الحرة تتفاعل مع الجزيئات البيولوجية و تؤثر علي تركيب الخلايا التي تسبب العديد من الأمراض مثل: السرطان- أمراض الكبد- أمراض الشيخوخة.....ألخ.
في هذة الدراسة تم تقدير للنباتات المختارة المحتوي الكلي للفينولات - 1و1 ثنائى فينايل-2 بكريل هيدرازيل (DPPH·) – نشاط الإرتباط بالشق الحر الهيدروكسيل (OH·) – أكسدة اللبيدات.
تم تقدير المحتوي الكلي للفينولات للنباتات المستخلصة بواسطة الإيثانول كحامض جاليك و كذلك تم تقيم قدرتها كمضادات الأكسدة بأستخدام تركيزات مختلفة من هذة النباتات المستخلصة (25و 50و 100 ميكروجرام/ مل) بإستخدام ثلاث طرق معملية : تثبيط الشق الحر (DPPH·) و الشق الحر الهيدروكسيل (OH·) و اكسدة اللبيدات في مستخلص الميتوكندريا بطريقة كبريتات الحديديك/ حامض الأسكوربيك.
أثبتت النتائج أن كل النباتات المستخلصة بواسطة الإيثانول تزداد قدرتها كمضادات الأكسدة بزيادة تركيز هذةالمستخلصات. المستخلص الإيثانولي لأوراق الجوافة وقلف القرفة و قشور الرمان أدت إلي حدوث تثبيط معنوي لأكسدة اللبيدات بطريقة كبريتات الحديديك/ حامض الأسكوربيك وتثبيط تكسير ديوكسي ربيبوز.
تعتبرأوراق الجوافة أفضل النباتات المستخلصة كمضادات الأكسدة وكلا من المستخلص الإيثانولى لأوراق الجوافة وقلف القرفة و قشور الرمان مضادات الأكسدة طبيعية وبذلك تعمل كعامل حماية طبيعي ضد عوامل الأكسدة المختلفة.
تم التعرف علي التركيب الكيماوي للمستخلص الإيثانولي بواسطة جهاز التحليل الكروموتوجرافي الكتلي (GC-Mass) لكل من أوراق الجوافة و قشور الرمان حيث إحتوي كلا منهما علي 19, 23 مركب علي التوالي.

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