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Evaluation of antioxidant potential of 124 Egyptian plants with emphasis on the action of *Punica granatum* leaf extract on rats

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Abstract: There is currently an upsurge of interest in phytochemicals as a new source of natural antioxidants to be used in foods and pharmaceutical preparations to replace synthetic antioxidants, which are being restricted due to their potential health risks and toxicity. The chloroform and methanolic leaf extracts of 124 Egyptian plant species belonging to 56 families were investigated and compared for their antioxidant activity by DPPH scavenging assay. Among the 124 plant species tested, 18 exhibit extremely high antiradical activity (> 80% inhibition). The methanolic leaf extract of the promising plant species (18 plants) were further subjected to determine their IC_{50} values and total phenolic and flavonoid contents. The IC_{50} ranged from 18.68 to 30.97 µg/ml, while total phenolic and flavonoid contents ranged from 162.06±4.55 to 242.26±18.65 mg Tannic acid equivalent (TAE)/g extract and from 32.56±1.52 to 157.96±5.85 mg Rutin equivalent (RE)/g extract, respectively. Correlation coefficient (r) between the DPPH radical scavenging activity (IC_{50}) and the total phenolic and flavonoid contents (r=0.63 and 0.51, respectively) suggested that phenolics and flavonoids in the extracts were partly responsible for the antiradical activities. The edibility and safety limits of leaf methanolic extract of *Punica granatum*, which exerted the highest antioxidant activity was assessed by using experimental albino rats. The results indicated that this extract was edible and safe at concentration of 20, 40 and 60 ppm within 9 weeks.

Keywords: Natural antioxidants, DPPH, total phenolic content, total flavonoid content, free radical scavenging activity, plant extracts, rats

Introduction

Free radicals may be defined as any chemical species that are capable of existing with one or more unpaired outer shell electrons. They are extremely reactive and generally highly unstable (Martinezy-Cayuela, 1995). They are produced endogenously during cellular metabolism in all forms of aerobic living system, in addition to exogenous sources such as environmental pollutants, drug, radiation, and pathogens (Ansari, 1997). The imbalance between production of reactive oxygen species (ROS) like O_2^-, H_2O_2, OH^-, ROO^- and the capacity of the normal detoxification system in favor of the oxidants lead to oxidative stress, which itself lead to cellular damage caused by the interaction of ROS with cellular constituents. Tissue damage resulting from oxidative stress has been implicated in the pathology of a number of disorder diseases such as cancer, inflammatory joint disease, cardiovascular diseases, cataract and could play a role in neurodegenerative diseases and ageing processes (Steer *et al*., 2002).

The term antioxidant refers to a compound that can delay or inhibit the oxidation of biomolecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent damage done to the body's cell by oxygen, i.e. ROS. Epidemiological studies have shown that many phytonutrients particularly phenolic compounds might protect the human body against damage by ROS.The consumption of fruits and vegetables which contain a wide variety of antioxidant phenolic compounds was reported to have potential health benefits (Sumino *et al*., 2002).

In addition, antioxidants have been widely used in food industry to prolong the shelf life. Synthetic antioxidants such as butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) are widespread food additives used to preserve against deterioration; however their use is increasingly restricted due to their potential health risks and toxicity Moreover, there is a growing awareness among consumer regarding food additives safety (Valentao *et al*., 2002). These reasons may explain the interest in examining plant extracts as a source of safe, cheap and effective antioxidants and the growing interest in nutraceuticals.

Plant derived antioxidants exert their effects by enhancing the level of antioxidant enzymes such as

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superoxide dismutase or by lowering the levels of lipid peroxides in the blood or liver (Usoh *et al*., 2005). It is recognized that antioxidant (mainly phenolic) compounds from plant extracts can act by either free radical scavenging, singlet oxygen quenching, chelating of transitional metal such as iron, as well as a reducing agents and activator of antioxidative defense enzyme systems to suppress radical damage in biological system (Lodovici *et al*., 2001).

Phenolic compounds are secondary plant metabolites that possess on their structure a benzenic ring substituted by, at least, one hydroxyl group (Manach *et al*., 2004). These compounds are ubiquitously distributed in the plant kingdom and exhibit a wide range of pharmacological and medicinal properties, including anti-inflammatory, anti-carcinogenic, vasodilatory actions (Middleton *et al*., 2000); these protective effects have been mostly ascribed to their free radical scavenging, metal chelating and antioxidant action.

In the recent years a large number of endemic plant species have been screened as a viable source of phenolic antioxidants (Wong *et al*., 2006; Tawaha *et al*., 2007; Annan and Houghton 2008; Dall'Acqua *et al*., 2008, Borneo *et al*., 2009; Rohman *et al*., 2010).

The aim of this study was to evaluate *in vitro* antioxidant potential of the leaves of 124 plant species growing in Egypt. The effect of three concentrations of methanolic extract which showed the highest antioxidant potential i.e. *Punica granatum* leaf extract on body weight, liver and kidney functions of albino rats was also investigated to assess the safety limits of this extract.

Materials and Methods

Chemicals

All chemicals and reagents used in this study were of analytical grade purchased from Sigma Chemical Co. (St.Louis,MO,USA) Aldrich Chemical Co. (Steinehein, Germany), BDH(Dorset-England) or Fluka Chemie Co. (Buchs, Switzerland).

Plant material

A total of 124 leaf samples of plant species belonging to 56 families were collected during April and May 2008 from El-Shrouk farm, Cario-Alexandria desert road at 72 km north of Cario. The botanical identification of the collected plants was authenticated by Dr. T. Labeb, Herbarium of Orman garden, Horticulture Research Institute, Giza, Egypt. A voucher specimen of each plant was deposited in the Herbarium of the Biochemistry Department, Faculty of Agriculture, Fayoum University. The leaves were cleaned, air-dried in the shade, and then powdered by laboratory mill to 24 mesh. Powdered materials were maintained in an air tight container at room temperature (28±2ºC), and protected from light until use.

Extraction

A known amount of air dried powdered leaves of each plant sample was extracted at room temperature (28±2ºC) with chloroform then with methanol. This procedure was repeated at least five times until each organic solvent became colorless. The obtained extracts were filtered over Whatman No.1 filter paper and the combined extract (filtrate) was evaporated to dryness under vacuum at 45ºC using a rotary evaporator. The dried extract (residue) obtained from each plant material was stored in a desiccators at 4ºC until further use.

DPPH free radical scavenging assay

Free radical scavenging activity was measured by the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, according to the method described by Brand-Williams *et al*, (1995). Briefly, a 2.0 ml of methanolic solution of each extract at concentration of 50 µg/ml was added to a 2.0 ml solution of DPPH (25 mg/l) in methanol, and the reaction mixture was shaken vigorously. After incubation at room temperature (28±2ºC) for 30 min, the absorbance (A) of DPPH for control and samples was determined by Cecil 3000 spectrophotometer at 517nm, and the radical scavenging activity of each extract was expressed as percentage inhibition of free radical DPPH.

$$\% \text{ inhibition (\% Anti-radical activity)} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

Triplicate measurements were taken and mean values calculated. IC_{50} (concentration of each sample at 50% inhibition) was obtained by linear regression analysis of the dose response curve, plotted between % inhibition and concentrations (2.5-100 µg/ml) L-Ascorbic acid was used as a positive control.

Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu reagent as described by Yu *et al*. (2002). Two hundred micro liters of each sample were mixed with 500 µl of the Folin-Ciocalteu reagent and 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. After 2 h of incubation at room temperature, the absorbance of the resulting blue-colored solution was measured at 765 nm. The assay was carried out in triplicate and the mean values were calculated.

The concentration was determined from the standard curve prepared using serial concentrations of standard tannic acid solution. Total phenolic contents in plant extracts were expressed as mg tannic acid equivalent (TAE)/g plant extract.

Determination of total flavonoid content

Total flavonoid content was determined using the method described by Lamaison and Carnet, 1990 as follows: Two hundred micro liters of each sample were transferred to a test tube and evaporated to dryness. To the residue, 5 ml of 0.1 M $AlCl_3$ were added and shaken. The intensity of the developed color was measured at 415 nm after 40 min versus the prepared blank. Triplicate measurements were taken and mean values calculated. Rutin was used as standard for the calibration curve. Total flavonoid content was expressed as mg rutin equivalent (RE) /g plant extract.

Effect of the methanolic extract of Punica granatum *on albino rats*

The effect of orally administrating of *Punica granatum* methanolic leaf extract on body weight, liver, and kidney function tests of albino rats was studied as follows:

Animals and diets

Twenty female albino rats with body weight of 95-101 g were obtained from the Faculty of Agriculture, Menia University, Egypt, and were acclimated for one week prior to the experiment. They were housed in groups of five each in universal polypropylene cages at room temperature (25±2◦C) and at a photoperiod 12 h/day. Animals were fed on standard laboratory chow (El-Nasar Lab. Chem. Co., Egypt) and water *ad libitum* till the end of the experiment (9 weeks).

Experimental design

The rats were divided into four experimental groups. Group I rats (control group) orally received 1 ml H_2O/day. Group II rats orally received daily 20 ppm methanolic *Punica granatum* extract (in 1 ml H_2O). Group III rats orally received daily 40 ppm methanolic *Punica granatum* extract (in 1 ml H_2O). Group IV rats orally received daily 60 ppm methanolic *Punica granatum* extract (in 1 ml H_2O). Body weight (BW) was recorded weekly during the experimental period (9 weeks) and food intake was measured daily during the adaptation and the experimental periods.

At the end of the experimental period (9 weeks) animals were fasted overnight, blood samples were withdrawn by a fine capillary glass tubes from the orbital plexus vein. The blood was collected in EDTA containing tubes and left at room temperature. Plasma was obtained by centrifugation at 3000 rpm for 15min and then stored at -20◦C until analysis. Liver and kidney organs were excised, rinsed in chilled saline solution, and then plotted on filter paper, weighed separately to calculate the absolute organs weight.

Analytical procedures

Plasma enzyme activity of aspartate aminotransferase (AST; E.C.2.6.1.1.) and alanine aminotransferase (ALT; E.C.2.6.1.2.) activities were measured according to the method described by Reitman and Frankel, 1957. Total protein, albumin, bilirubin and urea were determined according to the methods described previously (Gornal *et al*., 1948; Doumas *et al*., 1971; Fawcett and Soctt, 1960), using enzymatic colorimetric procedures Kits from Bio-Diagnostic Co., Egypt. Globulin was determined by difference between total protein and albumin.

Statistical analysis

Statistical Package for Social Science (SPSS) window's version (10, USA) was used for analysis of the data.

Results and Discussion

DPPH radical scavenging activity

The chloroform and methanol leaf extracts of 124 plant species extracts at 50 µg/ml were tested for antioxidant activity using the DPPH assay. The results were expressed as percentage inhibition (Table 1). As shown in Table 1, there is a wide range of free radical scavenging activity of the chloroform and methanolic extracts of the plant species analyzed. The values ranged from 0.5 to 49% and from 3 to 96% for chloroform and methanolic extracts, respectively. The variation of the free radical scavenging activity may be due to the differences in their secondary constituents (Sudjaroen *et al*., 2005).

According to the data obtained the plant species were classified into four categories on the basis of free radical scavenging activity low antioxidant power (<25% inhibition), medium (25-50% inhibition), high (50-80% inhibition) and extremely high (>80% inhibition). The number of plants in each of the low, medium, high and extremely high antioxidant power categories was 44, 52, 11 and 18 respectively.

The extremely high antioxidant power category (18 plant species) was further subjected to determine their IC_{50} values, which is defined as the concentration of substrate at 50% inhibition (Han *et al*., 2004). The IC_{50} values (µg/ml) of DPPH radical scavenging

Table 1. 124 Egyptian plants studied and free radical scavenging activity of their chloroform and methanolic leaf extracts

Plant Species	Family	% Antiradical activity Chloroform extract (50 µg/ml)	Methanol extract (50 µg/ml)
Adhatoda vasica	Acauthaceae	3	13
Dracaena marginata	Agavaceae	15	13
Yucca desmettiana	"	3	6
Polianthes tuberose	"	8	12
Alternanthera versicdor	Amaranthaceae	0.5	71
Amaranthus tricolor	"	1	0.0
Crinum longifolium	Amaryllidaceae	6	21
Schinus terebentifolius	Anacardiaceae	15	12
Foeniculum vulgare	Apiaceae	46	16
Carissa grandiflora	Apocynaceae	2	0.0
Thevetia nariifolia	"	26	29
Plumeria alba	"	28	15
Tabernaemontana divaricata	"	40	44
Vinca rossea	"	9	48
Nerium oleander	"	19	31
Anthorium scherzerianum	Araceae	14	37
Sciadophyllum pulchrima	Araliaceae	1.5	18
Schefflera actinophylla	"	3	94
Schefflera arboricola	"	2	0.0
Cryptostegia grandiflora	Asclpiadaceae	0.0	35
Chrysanthemum Red	Asteraceae	15.5	35
Santolina chamaecyparissus	"	11	46
Tecoma capensis	Bignoniaceae	0.0	55
Spathodea tilotica	"	19	85
Kigelia pinnata	"	3	0.0
Jacarandas acutifolia	"	18	55
Tecomaria capensis	"	9	90
Bombax malabaricum	Bombacaceae	2	96
Cordia sebestena	Boraginaceae	15	42
Poinciana regia	Caesalpiniaceae	15	15
Lonicera japonica	Caprifoliaceae	5	16
Euonymus japonica	Celastraceae	3	66
Conocarpus erectusl	Combretaceae	15	45
Terminalia arjuna	"	27	42
Tradescantea zebrina	Commelinaceae	1	16
Tradescantea spp	"	44	0.0
Gazania splendens	Compositae	0.0	89
Chrysanthemum frutesence	"	0.0	87
Aspidistra lurida	Convallariaceae	0.0	29
Thuja orientalis	Cupressaceae	0.0	71
Cupressus sempervirens	"	6	65
Cypressus macrocarpa	"	3	49
Cyperus alternifolius	Cyperaceae	2	31
Elaeagnus macrophylla	Elaeagnaceae	45	8
Jatropha integerrima	Euphorbiaceae	13	19
Jatropha multifidal	"	47	17
Euphorbia splendens	"	5.5	31
Acalypha marginata	"	22	89
Sesbania aegyptiaca	Fabaceae	17	43
Pithecellobium dulce	"	11	23
Pelargonium zonal	Geraniaceae	12	32
Pelargonium oderatissimum	"	9	89
Rosmarinus officinalis	Lamiaceae	28.6	31
Salvia officinalis	"	45	49
Lavandula angustifolia	"	2.6	36
Laurus nobilis	Lauraceae	49	89
Cordyline fruticosa	Laxmamiaceae	18	21
Bauhenia variegata	Leguminosae	18	94
Cassia didymobotrya	"	19.5	12
Cassia fistula	"	21.6	45
Sanseviera guineenses	Liliaceae	23.5	18
Ruscus hyphoglossum	"	0.0	42
Phalangium variegata	"	3	17
Asparagus setaceus	"	5	15
Asparagus plumosus	"	0.0	16
Budleja hybrida	Loganiaceae	10	50
Caesalpinia pulcherrima	"	25	27
Coratonia siliqua	"	1.5	40
Albezzia anthelmintica	"	20	49
Parkinsonia aculeata	"	17	9
Lagerstroemia indica	Lythraceae	28	89
Hibiscus mutabilic	Malvaceae	0.0	40
Althaea rosea	"	19	58
Khaya senegalensis	Meliaceae	13	86
Melia azedarach	"	2	32
Calliandra haematocephala	Mimosaceae	10	23
Albezzia stipulata	"	1	18
Dracaena fragrans	Moraceae	15	33
Morus alba	"	14	40
Ficus nitida	"	5	25
Ficus benjamina	"	2	3
Ficus elastica Var decora	"	6	8
Ficus hawaii	"	3	25
Ficus ali	"	1	3
Ficus relegiosa	"	3	24

Plant Species	Family	% Antiradical activity Chloroform extract (50 µg/ml)	Methanol extract (50 µg/ml)
Ficus elastica	"	11.5	41
Ficus enfictoria	"	3.5	31
Ficus Natasia	"	5	16
Moringa pterygosperma	Moringaceae	15	30
Myoporum pictum	Myoporaceae	42.7	26
Eucalyptus rostrata	Myrtaceae	9	90
Eugenia uniflora	"	13	16
Myrtus Communis	"	26.6	90
Caliistemon lanceolatus	"	23	78
Bougainvillea spectabilis	Nyctaginaceae	12	38
*Bougainvillea "pixie-pink"**	"	22	39
Bougainvillea glabra var. sanderiana a	"	15.6	0.0
Bougainvillea glabra "variegata"	"	20	50
Narcissus tazetta	Oleaceae	22	40
Jasminum primulinum	"	6	30
Jasminum grandiflorum	"	39	52
Ligustrum ovalifolium	"	9	11
Jasminum sp	"	45.7	34
Araucaria excelsa	Pinaceae	22	43
Pittosporum tobira var.variegata	Pittosporaceae	18.6	54
Pittosporum tobira	"	11	9
Grass sp	Poaceae	5	30
Nephrlepis bostoniensis	Polypodiaceae	16	43
Punica granatum	Punicaceae	9	96
Rosa spp	Rosaceae	26.6	27
Rosa banksiae	"	25	42
Didonia viscose	Sapindaceae	0.0	90
Hydrangea Red	Saxifragaceae	4	29
Leucophyllum frutescens	Scrophulariaceae	16.4	31
Russelia juncea	"	17	30
Datura arborea	Solonaceae	17	56
Cestrum diurnum	"	30	20
Sterculia diversifolia	Sterculiaceae	14	88
Brachichton acerifolium	"	16	22
Strelitzia reginae	Streliziaceae	32	45
Duranta repens	Verbenaceae	16	44
Lantana camara	"	12	32
Lantana montevidensis	"	11	30
Vitex trifolia"purpurea"	"	22	89

activity from dried methanolic extracts of these plant species and the positive control (Ascorbic acid) are presented in Table 2. As shown in this Table, *Punica granatum* and *Bombax malabaricum* showed the highest DPPH radical scavenging activity (the lowest values of IC_{50} i.e., 18.68 and 18.80 µg/ml, respectively) while the plant with lowest DPPH scavenging activity (the highest value of IC_{50} i.e., 30.97) was *Spathodea tilotica*.

Between those high and low values a narrow range of antioxidant abilities were obtained among the 18 tested plant species. The DPPH radical scavenging effect of the positive control i.e. ascorbic acid was higher (IC_{50} =11.5 µg/ml) than all the 18 plant species studied. The IC_{50} values of the analyzed plant species were roughly from 1.62 to 2.69 times lower than ascorbic acid. However we should keep in mind that those of high DPPH scavenging activity value obtained for ascorbic acid is because we performed the assay on highly purified reference standard and not in complex materials such as that of plant species analyzed. This implies that although there are plants with good antioxidant abilities further concentration or purification is needed to achieve better antioxidant capacities.

In the recent years various plant species have been tested as botanical antioxidants using DPPH assay (Wong *et al*., 2006; Annan and Houghton 2008; Dall'Acqua *et al*., 2008; Borneo *et al*., 2009; Rohman *et al*., 2010). These species exhibited a broad range of antioxidant activities. The highest antioxidant activity has been reported for *Rupus ulmifolius*, IC_{50} values 5.1 µg/ml (Dall'Acqua *et al*., 2008), whereas the lowest antioxidant was reported for *Thelesperma megapotamicam*, IC_{50} values 2000 µg/ml (Borneo *et al*., 2009).

Total phenolics and flavonoids contents

The systematic literature collection, pertaining to this investigation indicates that the plant phenolics constitute one of the major groups of compounds acting as primary antioxidant or free radical terminators. Therefore, it is worthwhile to determine their total amount in plants chosen for the study. Flavonoids one of the most diverse and widespread group of natural compounds, are likely to be the most important natural phenolics due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties (Kahkonen *et al*., 1999). Therefore the contents of flavonoids is also determined.

Total phenolics content of methanolic extracts (TPC) of the 18 promising plant species were expressed as tannic acid equivalent (TAE) in milligrams per gram extract. As shown in Table 2, the TPC ranged from 162.06 to 242.26 mg TAE/g extract. *Punica granatum* and *Myrtus communis* showed the highest TPC of 242.26 and 241.56 mg TAE/g extract,

while *Vitex trifolia* "purpurea" was the lowest one in TPC (162.02 mg TAE/g extract).

The total flavonoid content (TFC) of the methanolic extracts of the promising plant species was expressed as milligram rutin equivalents (RE) per gram extract and are presented in Table 2.

Table 2. Total phenolic and total flavonoid contents of leaf methanolic extracts of the promising antioxidant plant species

Total flavonoids (mg RE/g extract)	Total Phenolic (mg TAE/g extract)	IC_{50} (µg/ml)	Family	Plant Scientific name
54.360 ±5.070^{m}	242.26 ±18.65^{a}	18.68	Punicaceae	*Punica granatum*
157.96 ±5.850^{a}	214.06 ±5.050^{abc}	18.80	Bombacaceae	*Bombax malabaricum*
32.560 ±1.520^{o}	220.86 ±4.750^{ab}	19.23	Araliaceae	*Schefflera actinophylla*
109.76 ±0. 850^{fg}	226.00 ±7.140^{ab}	19.36	Leguminosae	*Bauhenia variegata*
67.760 ±9.500^{l}	220.26 ±33.85^{ab}	20.73	Myrtaceae	*Eucalyptus rostrata*
113.66 ±1.050^{e}	208.26 ±41.65^{abcd}	21.05	Sapindaceae	*Didonia viscose*
126.96 ±1.150^{d}	241.56 ±11.75^{a}	21.53	Myrtaceae	*Myrtus Communis*
111.06 ±5. 500^{ef}	226.40 ±23.02^{ab}	22.55	Bignoniaceae	*Tecomaria capensis*
135.66 ±0. 550^{c}	162.06 ±4.550^{f}	22.94	Verbenaceae	*Vitex trifolia"purpurea"*
48.06 ±0. 350^{n}	208.86 ±10.95^{abcd}	23.57	Compositae	*Gazania splendens*
107.06 ±0. 450^{gh}	223.16 ±10.85^{ab}	23. 74	Lythraceae	*Lagerstroemia indica*
71.36 ±0. 650^{k}	177.16 ±6.250^{def}	24.57	Euphorbiaceae	*Acalypha marginata*
88.76 ±0. 850^{j}	205.16 ±9.150^{abcde}	25.30	Lauraceae	*Laurus nobilis*
104.96 ±0. 550^{h}	180.66 ±12.45^{def}	26.00	Geraniaceae	*Pelargonium oderatissimum*
111.06 ±0. 550^{ef}	171.66 ±2.450^{ef}	27.32	Sterculiaceae	*Sterculia diversifolia*
69.06 ±0. 250^{kl}	208.66 ±8.650^{abcd}	27.83	Compositae	*Chrysanthemum frutesence*
149.36 ±0. 550^{b}	215.06 ±43.05^{abc}	29.61	Meliaceae	*Khaya senegalensis*
129.96 ±0. 750^{d}	197.26 ±6.550^{bcde}	30.97	Bignoniaceae	*Spathodea tilotica*
		11.5		*Ascorbic acid*

Each value is presented as mean ±SD (n=3). Means within each column with different letters (a-m) differ significantly ($p<0.05$).

The TFC ranged from 32.56 to 157.96 mg RE/g extract for *Bombax malabaricum* and *Schefflera actinophylla*. The 18 analyzed plant species have the same geographic origin and grow in the same natural condition; nevertheless the plants belonging to different families. It is well known that the amount of total phenolics vary with respect to families and varieties (Kahkonen *et al*., 1999; Djeridane *et al*., 2006).

The results in Table 2, also indicated that *Punica granatum* had the best DPPH radical scavenging activity and also the highest total phenolics content. However, as previously mentioned, *Myrtus communis* had higher phenolics content (241.56 mg TAE/g extract) than *Bombax malabaricum*, *Schefflera actinophylla*, *Bauhenia variegata*, *Eucalyptus rostrata* and *Didonia viscose* (214.06 ,220.86 , 226.0, 220.26 and 208.26 mg TAE/g extract ,respectively), but these plant extracts exhibited high DPPH radical scavenging activity than *Myrtus communis*. Also *Vitex trifolia* had lower phenolics content (162.06 mg TAE/g extract) than *G.splendens*, *L.indica*, *A. marginata*, *L. nobilis*, *P. oderatissium*, *S.diversifolia*, *C. frutesence*, *K. senegalensis* and *S. titotica* (208.86, 223.16, 177.16, 205.16, 180.66, 171.66, 208.66, 215.06 and 197.26 mg TAE/g extract respectively, however it had high DPPH radical scavenging activity. Folin assay gives a crude estimate of the amount of phenolic compounds present in an extract. It is not specific to polyphenols but many interfering compounds may react with the reagent, giving elevated apparent phenolic concentrations (Singleton *et al*., 1999). Moreover, various phenolics compounds respond differently in this assay, depending on the number of phenolic groups they have and total phenolics content does not incorporate necessarily all the antioxidant that may be present in an extract (Tawaha *et al*., 2007).

Correlation between DPPH radical scavenging activity and total phenolic and flavonoid compounds

The correlation coefficient (r) between the free radical scavenging activity and both the total phenolic and the total flavonoid contents of the promising plants was determined (Figure1). The correlation coefficient between the scavenging activity obtained from DPPH assay and the phenolic content was 0.63 (Figure 1A) whereas with total flavonoid contents has correlation coefficient of 0.51 (Figure 1B). These results suggested that phenolic and flavonoid compounds contributed of 63.0% and 51.0% to free DPPH radical scavenging of the analyzed plant extracts. Also, it can be stated that scavenging effects is not limited to phenolic and flavonoid compounds. The activity also comes from the presence of other antioxidant secondary metabolites in the extracts which directly or indirectly contribute to the activity.

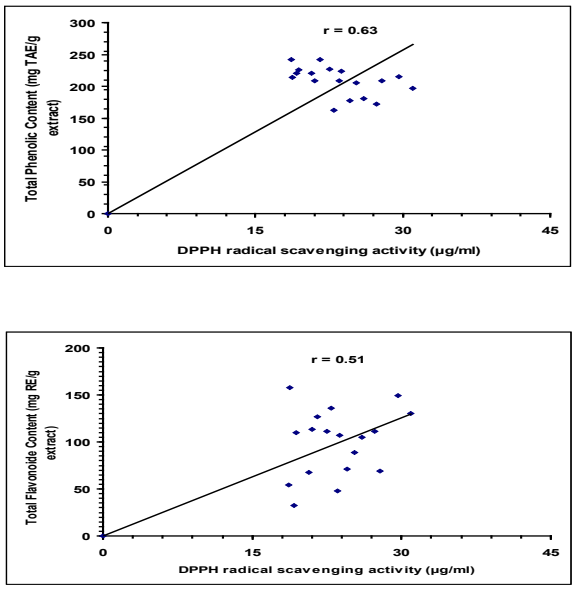

Figure 1. Correlation between free DPPH radical scavenging activity (IC50) and total phenolic contents (A) and with total flavonoid contents (B)

Table 3. Effect of different levels of orally methanolic extract administration of Punica granatum on body weight and liver and kidney organs weight

Group No.	Initial body Weight (g)	Final body Weight (g)	Liver Weight (g)	Kidney Weight (g)	Weight gain (g)	Food consumption (g/day)	Food efficiency
I (control)	100.8±12.7	192.0±11.27	6.23±0.41	1.23±0.14	91.2±11.27	82.10	1.11
II (20mg/kg)	95.0±0.0	195.67±0.58	7.09±0.26	1.34±0.22	100.7±0.58	86.30	1.17
III (40mg/kg)	99.0±2.24	196.0±1.73	6.58±.0.5	1.31±0.07	97.00±2.54	85.04	1.14
IV (60mg/kg)	99.0±2.24	196.0±1.73	7.02±0.41	1.28±0.09	97.00±2.54	91.36	1.06

Values are expressed as mean ± S.D. (n=5).

Table 4. Effect of different levels of orally methanolic extract administration of Punica granatum on liver and kidney functions in plasma of female albino rats

Group No.	ALT(GPT) (U/L)	AST(GOT) (U/L)	T.Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Urea (mg/dl)	Total bilirubin (mg/dl)
I (control)	42.9±2.7	62.7±7.2	5.902±0.62	3.689±0.20	2.213±0.67	79.076±24.69	0.567±0.16
II (20mg/kg)	42.6±3.2	61.38±6.2	5.408±0.54	3.775±0.84	1.633±0.79	78.743±23.17	0.308±0.03
III (40mg/kg)	44.1±4.6	59.4±9.6	5.845±0.74	4.087±0.64	1.758±0.97	64.831±15.24	0.518±0.31
IV (60mg/kg)	42.6±3.4	62.92±7.3	5.55±0.50	3.90±0.57	1.654±0.81	65.423±21.75	0.254±0.10

Values are expressed as mean ± S.D. (n=5).

These data are in accordance others (Ou *et al*., 2003; Rohman *et al*., 2010; Cox *et al*., 2010) who have reported that there is no correlation between the content of these main antioxidant compounds and the radical scavenging activity, whereas many studies indicate a linear relationship between these two variables (Djeridane *et al*., 2006; Tawaha *et al*., 2007; Borneo *et al*., 2009).

Effect of leaf methanolic extract of Punica granatum on albino rats

In order to assess the edibility and safety limits of leaf methanolic extract of *Punica granatum* that, exerted the highest antioxidant activity. Three levels of methanolic extract preparation, including 20, 40 and 60 mg / kg body weight (group II, III and IV respectively) were used in this experiment in addition to control (group I). The obtained results are shown in Table (3) and (4). No significant differences were noted in the mean value of body weight and organs weight among different experimental groups (Table 3). Results obtained in Table 4 showed no significant changes in liver enzymes activity ALT or AST in treated groups compared with control rats. Also, no significant effects were observed in plasma bilirubin, total protein, albumin and globulin in treated groups corresponding to control group. Meanwhile, slight decrease in urea was shown in the different treated groups (Table 4), but this slight decrease is not significant as compared with control group. These results indicated that orally administration of the three concentrations of leaf methanolic extract of *Punica granatum* has no negative effect on body weight or liver and kidney function tests of female albino rats. The edibility of the methanolic extract was observed as the food consumption and so body weight was increased. The results of the experimental animals indicated that the three concentrations of leaf methanolic extract of *Punica granatum* was safe and edible.

Conclusion

In conclusion, this study suggests that some of the plant species studied may be a good source of natural antioxidants. Further studies are warranted for isolation and characterization of the active components of promising plant species as well as *in vivo* evaluation of toxicity of these active components in animals studies.

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