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ORIGINAL ARTICLES

Screening of Antioxidant and Cytotoxicity Activities of Some Plant Extracts from Egyptian Flora

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ABSTRACT

Antioxidants play an important role to protect the body from damage caused by free radical-induced oxidative stress. The antioxidant activities of 77 methanolic plant extracts were screened. This study is to submit some Egyptian plants to systematic antioxidant screening. The methanolic extracts of 77 plants were screened for their effect on 2, 2-Diphenyl-1-picryl-hydrazyl radical (DPPH) to determine their free radical scavenging activity. Extracts that gave 90% DPPH reduction or more were evaluated for total antioxidant, reducing power, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities. On subjecting the 77 methanolic extracts to DPPH, seven extract out of them gave 90% or more namely: *Lycium europium* L., *Liomoniastrum monopetalum* (L.) Boiss., *Varthemia candicans* (Delile) Boiss. *Jasonia candicans* (Delile) Botsch., *Phlomis floccosa* D. Don, *Lavandula coronopifolia* Poir., *Thuja orientalis* L. and *Areca catechu* L. Total antioxidant activity, Ferric reducing antioxidant power and superoxide radical scavenging activity of *Thuja oriental* and *Areca catechu* were more potent than L-ascorbic acid and BHT. Methanolic extract of *Liomoniastrum, Phlomis* has powerful Metal chelating activity while *Lycium* has potent scavenging activity against hydrogen peroxide. These extracts have low cytotoxicity effect on human tumor cell lines. The findings of this study support this view that some medicinal plants are promising sources of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. Further studies on these active methanolic extracts are required to isolate and identify fraction(s) or compound(s) responsible for their activity.

Key words: Antioxidant; Cytotoxicity; DPPH; FIC; FRAP; plant extracts; Superoxide scavenging, TBARS.

Introduction

Free radicals are a major cause of oxidative stress that may lead to DNA strand breakage, *gene* mutation and DNA-DNA and DNA-protein cross links. Free radicals are known to be a product of normal metabolism. When oxygen is supplied in excess or its reduction is insufficient, reactive oxygen species (ROS) such as hydroxyle (OH^-), superoxide (O_2^-), nitric oxide (NO), lipid peroxyle (Loo^-), radical and non-free radical species such as lipid peroxide ($LOOH^-$) and different forms of activated oxygen (Helliwelled et. al., 1999, Yildirim *et al*., 2000, Gulcin *et al*., 2002a). ROS are involved in an organism's vital activities including phagocytosis, regulation of cell proliferation, intracellular signaling and synthesis of biologically active compounds (Halliwell and Gutteridge, 1989; Miquel and Romano-Bosca, 2004). ROS have been implicated in several diseases including carcinogenesis, malaria, heart diseases, arteriosclerosis, diabetes and many other health problems related to ageing (Duh, 1998; Honda *et al*., 2004; Tanizawa, *et al*., 1992; Uchida, 2000). The role of ROS in the etiology and progression of several clinical manifestations has led to the suggestion that the antioxidants can be beneficial as prophylactic agents. Nevertheless, all aerobic organisms, including humans, have antioxidant defenses that protect against oxidative harm and repair damaged molecules.

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However, the natural antioxidant mechanisms can be insufficient, the supply of antioxidants through dietary ingredients, is of great interest for a healthy life (Duh, 1998; Espin *et al*., 2000; Greenwald *et al*., 2001; Scalbert and illiamson, 2000; Terao *et al*., 1994). A number of plants have been documented for their antimicrobial (Ahmad and Beg, 2001; Polambo and Semple, 2001) and antioxidant activities (Gajera *et al*., 2005).

Although significant progress has been made in antioxidant and cancer chemoprevention drugs, current drugs are ineffective and expensive. For these reasons the discovery of new drugs is an important and necessary strategy in improving chemotherapy. Since medieval times, plants have been the source of medicines for the treatment of diseases. Regardless of the availability of a wealth of synthetic drugs, plants remain even in the 21st century an integral part of the health care in different countries, especially the developing ones. The research in the areas of natural products and traditional medicine could play a significant role in the health care system of the developing countries. Natural products once served mankind as the source of all drugs and higher plants provided most of such therapeutic agents. Today, natural products (and their derivatives and analogues) still represent over 50% of all drugs in clinical use, with higher plant-derived natural products representing *ca*. 25% of the total **(**Brindley, 1994). In the current study seventy seven were collected native and cultivated plants growing in Egypt to assess their antioxidant potential by measuring their total antioxidant activity, reducing power, metal chelation, superoxide scavenging, hydrogen peroxide scavenging and radical scavenging activities to suppress the extent of lipid peroxidation and also tested for their cytotoxicity effect.

Materials and methods

Chemicals:

Ammonium thiocyanate was purchased from E. Merck. Ferrous chloride, ascorbic acid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2, 4-triazine (ferrozine), nicotinamide adenine dinucleotide (NADH), butylated hydroxy toluene (BHT), and trichloroacetic acid (TCA) were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Nitroblue tertazolium salt was purchased from Fluka Bio Chemica

Plant Material:

Plant material used in this work belonged to 77 species were collected randomly (Table 1). Native species were collected from Western Mediterranean coastal region while the cultivated taxa were obtained from herbarium of Zoo Garden and Orman Botanical Garden (Cairo, Egypt). Voucher specimens are deposited in the National Research Center (NRC-Plant Drug Discovery Herbarium), Dokki, Giza, Egypt. The identification of native plants was carried out by Loutfy Boulos, and the nomenclature is in accordance with Boulos (1999, 2000, 2002, 2005). The identification of cultivated plants was done by engineering Therese Labib, and the nomenclature follows Huxley *et al*., (1992).

Table 1: Plant materials.

Code	Plant name	Locality	Plant part
B1	Zygophyllum album L. f.	(WMCR): Burg El-Arab	A.P
B2	Haloxylon salicornicum (Moq.) Bunge ex Boiss.	WMCR: Burg El-Arab	A.P
B3	Artemesia monosperma Del.	WMCR: Burg El-Arab	A.P
B4	Suaeda aegyptiaca (Hasselq.)Zohary		A.P
B5	Pluchea dioscoridis (Conyza dioscorides)	WMCR:Burg El-Arab	A.P
B6	Cornulaca monacantha Delile	WMCR:Burg El-Arab	A.P
B 7	Lycium europium L.	WMCR:Mersa-Matruh	A.P
B 8	Annona cherimola Mill.	Orman Botanical Garden	Branches
B 9	Ononis vaginalis Vahl.	WMCR:Mersa-Matruh	A.P
B 10	Enarthrocarpus strangulates Boiss.	WMCR:El-Negeila	A.P
B 11	Ephedra aphylla Forssk.	WMCR: Wadi Halazin	A.P
B 12	Peganum harmala L.	WMCR: Burg El-Arab	A.P
B 13	Salsola kali L.	WMCR: El-Hammam	A.P
B 14	Carduncellus mareoticus (Delile) Hanelt	WMCR: Burg El-Arab	A.P
B 15	Salsola villosa Schult.	WMCR: Mersa Matruh	A.P
B 16	Deverra tortuosa (Desf.) DC.	WMCR: Wadi Halazin	A.P
B 17	Capparis spinosa L. var.inermis Turra	WMCR: Wadi Halazin	A.P
B 18	Aster squamatus (Spreng.) Hieron.	WMCR: Burg El-Arab	A.P
B 19	Marrubium alysson L.	WMCR: Mersa-Matruh	A.P

Table 1: Continue.

B 20	Malus sylvestris Mill	Zoo garden	A.P
B 21	Phragmites australis (Cav.) Trin. ex Steud. Arundo australis Cav.	WMCR: Burg El-Arab	A.P
B 22	Liomoniastrum monopetalum (L.) Boiss.	Zoo garden	A.P
B 23	Hippeastrum vittatum (L Her.)	Orman Botanical Garden	Leaves
B 24	Cedrela toona Roxb. ex Rottl.	Orman Garden	Leaves
B 25	Euphorbia paralias L.	WMCR: Mersa-Matruh	A.P
B 26	Withania somnfera (L.) Dunal.	Orman Botanical Garden	A.P
B 27	Alhagi graecorum Boiss.	Zoo garden	A.P
B 28	Lolium perenne L	WMCR: Wadi Habis	A.P
B 29	Salvia splendens F .sellow ex Roem &Schut	Orman Botanical Garden	branches
B 30	Varthemia candicans (Delile) Boiss.	WMCR: Wadi Halazin	A.P
B 31	Cassia bicapsularis L.	Zoo	A.P
B 32	Tamarix tetragyna Ehrenb.	WMCR: Burg El-Arab	A.P
B 33	Bougainvillea glabra Choisy	Orman Botanical Garden	A.P
B 34	Blackiella inflata (F. Mueller)	WMCR: Burg El-Arab	A.P
B 35	Delonix regia (Bojer) Raf.	Orman Botanical Garden	A.P
B 36	Thymeleae hirsuta L. Endler	WMCR: Alamein	A.P
B 37	Duranta repens var.variegata (Duranta plumeri) L.M.Bailey	WMCR: Wadi Halazin	A.P
B 38	Euphorbia denderoides L.	WMCR: Wadi Halazin	A.P
B 39	Cordia sinensis Lam.	zoo	Branches
B 40	Severinia buxifolia (Poir) Ten	Orman Botanical Garden	Leaves
B 41	Ailanthus altissima (Mill.) Swingle	Orman Botanical Garden	Branches
B 42	Phlomis floccosa D. Don	WMCR: Wadi Habis	Branches
B 43	Cordia holstii Gurke.	Zoo Garden	Leaves
B 44	Zygophyllum simplex L.	WMCR: Mersa-Matruh	Branches
B 45	Cordia mixa L.	Zoo Garden	Branches
B 46	Cereus jamacaru DC.	Orman Botanical Garden	A.P
B 47	Chamaecyparis lawsoniana (A.Murr.) Parl.	Orman Botanical Garden	Bark
B 48	Chrysanthemum cornoarium L.	WMCR: El-Negeila	A.P
B 49	Citharexylon quadrangulrais Jacq	Orman Botanical Garden	Branches
B 50	Gleditsia triacanthos L.	Orman Botanical Garden	Seed
B 51	Thuja orientalis L.	Zoo garden	A.P
B 52	Ammophila arenaria (L.) Link subsp. arundinacea H. Lindb.	WMCR: Ras El-Hekma	A.P
B 53	Arthrocnemum macrostachyum (Moric.) K. Koch	WMCR: El-Hammam	A.P
B 54	Phytolacca dioica L.	Orman Botanical Garden	Leaves
B 55	Asphodelus ramosus L.	WMCR: Wadi Habis	Root
B 56	Ammi majus L.	WMCR: Wadi Agiba	A.P
B 57	Astragalus trigonus DC.	WMCR: Sidi Abd El-Rahman	A.P
B 58	Achillea santolina L.	WMCR: El-Negeila	A.P
B 59	Areca catechu L.	Zoo garden	Fruits
B 60	Avena sterilis L. subsp. Sterilis	WMCR: Sidi Abd El-Rahman	A.P
B 61	Brassica tournefortii Gouan	WMCR: Wadi Habis	A.P
B 62	Cordyline terminalis L. Kunth	Zoo garden	Leaves
B 63	Carduncellus mareoticus (Delile)	WMCR: Burg El-Arab	A.P
B 64	Paronychia arabica (L.) DC.	WMCR: Wadi Abu El-Grouf,	A.P
B 65	Anacyclis alexandrinus Willd.	WMCR: Wadi Habis	A.P
B 66	Hordeum vulgare L.	WMCR: Wadi Agiba	A.P
B 67	Centaurea pumilio L.	WMCR: Ras El-Hekma	A.P
B 68	Crucianella maritima L.	WMCR: Ras El-Hekma	A.P
B 69	Cyperus alopecuroids (Rottb.)	WMCR: Burg El-Arab	A.P

Preparation of Plant Extracts:

Small quantities (0.5-3.0 kg) were collected from each plant for preliminary bioscreening.. Routine protection of natural plant constituents from denaturation or artifact formation during the extraction and concentration procedures was ensured to prepare the crude extracts (El-Menshawi, 2010). Whole plant or plant part was dried in a solar oven at 40ºC, ground and extracted with methanol (80%) at ambient temperature by percolation. Extracts were filtered and methanol was evaporated to dryness under reduced pressure and totally freed from water by freeze-drying and stored under freezing at -70ºC until use.

Methods:

Free Radical Scavenging Activity:

The free radical scavenging activity of extracts was measured using 1,1-diphenyl-2-picryl-hydrazil (DPPH$^{\bullet}$) adopting the method of Shimada *et al*,. (1992). 100µg/ml of different plant extracts were screened while the most potent extracts which showed more than 90% radical scavenging activity were assayed for other antioxidant testing assays. Briefly, 0.1mM solution of DPPH$^{\bullet}$ in methanol was prepared and then 1 ml of this solution was added to 3 ml of extract solution.

The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in Asys microplate reader. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The reaction was carried out with three replicates for each extract.

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 (Oktay *et al*., 2003).

Total Antioxidant Activity Determination:

Total antioxidant activity was measured according to the method described by Hsu *et al*. (2003). 0.2ml of peroxidase (4.4units/ml), 0.2ml of H_2O_2 (50µM), 0.2ml of ABTS(2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid, diammonium salt, 100 µM) and 1.0ml distilled water were mixed, and kept in the dark for 1h to form a bluish-green complex. After adding 1.0ml of extract, the absorbance at 734nm was measured. The total antioxidant activity was calculated as follows:

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

Reducing Power:

The reducing power of the different extracts was determined according to the method of Oyaizu (1986). Different concentrations of extracts (25, 50 and 75 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5ml, 0.2M, pH= 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloro acetic acid, TCA, (10%) was added to the mixture, which was then centrifuged for 10 min at 1000 × g. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in Shimadzu spectrophotometer. Ascorbic acid and butylated hydroxyl toluene were used as standard antioxidant compounds. Higher absorbance of the reaction mixture indicated greater reducing power.

Superoxide Anion Scavenging Activity:

Measurement of superoxide anion scavenging activity of the different extracts at different concentrations was based on the method described by Liu *et al*. (1997) with slight modifications (Gülçin *et al*., 2003c). Superoxide radicals are generated in phenazine methosulphate (PMS)–nicotinamide adenine dinucleotide (NADH) systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In this experiments, the superoxide radicals were generated in 3 ml of Tris–HCl buffer (16mM, pH= 8.0) containing 1 ml of NBT (50 µM) solution, 1 ml NADH (78 µM) solution and 1 ml sample solution were mixed. The reaction was started by adding 1 ml of PMS solution (10 µM) to the mixture. The reaction mixture was incubated at 25°C for 5min, and the absorbance at 560 nm in a Shimadzu spectrophotometer was measured against blank samples. L-Ascorbic acid was used as a control. Decrease in absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula

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

Where A_0 was the absorbance of the control (l-Ascorbic acid), and A_1 was the absorbance of extract and Ascorbic acid or BHT (Ye *et al*., 2000).

Metal Chelating Activity:

The chelating activity of ferrous ions by the extracts and standards was estimated applying the method of Dinis (Dinis *et al*., 1994). Briefly, extracts (25-75µg/ml) were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5mM ferrozine (0.2 ml) then the mixture was shaken vigorously and left standing at room temperature for ten minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm in a Shimadzu spectrophotometer.

The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula:

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

where A_0 was the absorbance of the control, and A1 was the absorbance in the presence of the sample of extracts and ascorbic acid or BHT. The control contains $FeCl_2$ and ferrozine (Gülçin *et al*., 2003a).

Scavenging of Hydrogen Peroxide:

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*. (1989). A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer. Extracts (25-75µg/ ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined after ten minute against a blank solution containing phosphate buffer instead of hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of the extracts and standard compounds was calculated using the following equation:

Percent scavenged $[H_2O_2] = [(A_0-A_1)\ A0] \times 100$

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of extract & ascorbic acid and BHT (Gülçin *et al*., 2003b).

Cytotoxicity Effect on Different Cell Lines (HePG2, MCF7, HCT116, A549):

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan(20).

Procedure:

All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were batch cultured for 10 days, then seeded at concentration of 10x103 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 h under 5% CO2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with a sample at concentration of 100 µg/ml (primary screening). Potent plant extracts from the primary bioassay were subjected to secondary screening for the determination of their LC50 and LC90 using different descending concentrations (100-50-25-12.5-6.25-3.125-0.78 and 1.56 µg/ml). Cells were suspended in RPMI 1640 medium(for HePG2, HCT116, A549) and DMEM media (for MCF7), 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine in 96-well flat bottom microplate at 37 ºC under 5% CO2. After 48 h of incubation, medium was aspirated, 40ul MTT salt (2.5µg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO2. To stop the reaction and dissolving the formed crystals, 200µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC. A positive control which composed of 100µg/ml of *Annona cherimolia* extract was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions (Hughes, *et al*., 1997).

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%.

The percentage of change in viability was calculated according to the formula:

(Absorbance of extract / Absorbance of negative control) -1) x 100

A probit analysis was carried for IC50 and IC90 determination using SPSS 11 program.

Statistical Analysis:

The results obtained in all analyses were expressed in mean ± SD (standard deviation). The levels of statistic significance ($p<0.05$) were calculated based on one-way ANOVA test for comparisons among means.

Results and discussion

As the trend of the future is moving towards functional food with specific healthy effects, the importance of antioxidant constituents of plant material in maintaining health and in protecting against coronary heart diseases and cancer is raising interest among scientists, food manufacturers and/or consumers (Kahkonen *et al*., 1999). The phenolic compounds have been reported to have multiple biological effects, including antioxidant activity. The presence of different antioxidant components in plant tissues makes it relatively difficult to measure each antioxidant component separately. Therefore, several methods have been developed, in recent years, to evaluate the antioxidant activity in biological samples (Kaur & Kapoor, 2002).

The antioxidant activities of plant extracts have been widely demonstrated (Sebranek *et al*., 2005) although the mechanism of such activity is not fully understood. Several explanations have been provided; the following are from these explanations: the sequestration of free radicals; hydrogen donation; metallic ion chelation; or even acting as substrate for radicals such as superoxide or hydroxyl (Al-Mamary, *et al*., 2002).

Determination of Antioxidant Activity Using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method:

The DPPH free radical does not require any special preparation and is considered a simple and very fast method for determining antioxidant activity. In contrast, DPPH can only be dissolved in organic media, especially in ethanol, which is an important limitation when interpreting the role of hydrophilic antioxidants (Wojdyło *et al*., 2007).

The radical scavenging capacity of different extracts tested using the 'stable' free radical (DPPH) was demonstrated in Table 2.

Table 2: The radical scavenging capacity of different extracts tested using the 'stable' free radical (DPPH) at 100µg/ml.

Code	Plant name	Mean DPPH Inhibition %(±SE)
B 1	Zygophyllum album L. f.	39.1 ±0.61
B 2	Haloxylon salicornicum (Moq.) Bunge ex Boiss.	68.4 ±0.17
B 3	Artemesia monosperma Del.	37.1 ±0.18
B 4	Suaeda aegyptiaca (Hasselq.)Zohary	37.5 ±2.6
B 5	Pluchea dioscoridis (Conyza dioscorides)	42.6 ±0.49
B 6	Cornulaca monacantha Delile	37.5 ±3.53
B 7	Lycium europium L.	96.5±0
B 8	Annona cherimola Mill.	39.1±0.13
B 9	Ononis vaginalis Vahl.	4.9±0.76
B 10	Enarthrocarpus strangulates Boiss.	23.3±0
B 11	Ephedra aphylla Forssk.	78.9±1.93
B 12	Peganum harmala L.	19.0±2.58
B 13	Salsola kali L.	49.0±0.68
B 14	Carduncellus mareoticus (Delile) Hanelt Carthamus mareoticus Delile	53.8±2.1
B 15	Salsola villosa Schult.	18.3±1.27
B 16	Deverra tortuosa (Desf.) DC.	15.7±1.89
B 17	Capparis spinosa L.	13.7±0.98
B 18	Aster squamatus (Spreng.) Hieron.	61.1±1.36
B 19	Marrubium alysson L.	9.9±1.5
B 20	Malus sylvestris Mill.	33.7±6.48
B 21	Phragmites australis (Cav.) Trin. ex Steud. Arundo australis Cav.	40.2±0.69
B 22	Liomoniastrum monopetalum L.	96.5±0.61
B 23	Hippeastrum vittatum L.	27.0±2.89
B 24	Cedrela toona Roxb.	35.6±0.49
B 25	Euphorbia paralias L.	21.4±0.67
B 26	Withania somnfera L.	16.2±0.67
B 27	Alhagi graecorum Boiss.	7.9±0.25
B 28	Lolium perenne L	18.7±0.61
B 29	Salvia splendens F .sellow ex Roem &Schut	19.8±0.38
B 30	Varthemia candicans (Delile) Boiss.	100±0
B 31	Cassia bicapsularis L.	9.2±0.96

Table 2: Continue.

B 32	Tamarix tetragyna Ehrenb.	82.7±2.49
B 33	Bougainvillea glabra Choisy	8.6±2.1
B 34	Blackiella inflata (F. Mueller)	3.8±0.42
B 35	Delonix regia (Bojer) Raf.	83.5±7.9
B 36	Thymeleae hirsuta L.	72.8±11.91
B 37	Duranta repens var.variegata (Duranta plumeri) L.M.Bailey	33.7±1
B 38	Euphorbia denderoides L. Endler	55.1±1.76
B 39	Cordia sinensis Lam	15.3±3.08
B 40	Severinia buxifolia (Poir) Ten	17.1±0.91
B 41	Ailanthus altissimoa (Mill.) Swingle	61.3±3.29
B 42	Phlomis floccosa D.Don	93.5±0.22
B 43	Cordia holstii Gurke	23.7±1.73
B 44	Zygophyllum simplex L.	0.0±0.0
B 45	Cordia mixa L.	9.0± 0.32
B 46	Cereus jamacaru DC.	8.4±0.18
B 47	Chamaecyparis lawsoniana (A.Murr.) Parl.	8.4± 0.18
B 48	Chrysanthemum cornoarium L.	52±9.27
B 49	Citharexylon quadrangulrais Jacq	17.3±1.28
B 50	Gleditsia triacanthos L.	20.6±1.88
B 51	Thuja orientalis L.	96.4±2.37
B 52	Ammophila arenaria (L.) Link subsp. arundinacea H. Lindb.	3.89±0.86
B 53	Arthrocnemum macrostachyum (Moric.) K. Koch	3.4±0.88
B 54	Phytolacca dioica L.	21.3±1.22
B 55	Asphodelus ramosus L.	38.2±3.07
B 56	Ammi majus L.	9.4± 0.3
B 57	Astragalus trigonus DC.	0.0±0.0
B 58	Achillea santolina L.	35.9±8.09
B 59	Areca catechu L.	100±0.0
B 60	Avena sterilis L.	14.7±1.2
B 61	Brassica tournefortii Gouan	30.5±1.56
B 62	Cordyline terminalis L	10.5±1
B 63	Carduncellus mareoticus (Delile)	53.8±2.1
B 64	Paronychia arabica (L.) DC.	73.1±0.95
B 65	Anacyclis alexandrinus Willd.	0.0±0.0
B 66	Hordeum vulgare L.	11.7±1.6
B 67	Centaurea pumilio L.	5.9±0.69
B 68	Crucianella maritima L.	43.0±0.13
B 69	Cyperus alopecuroids (Rottb.)	85.3±10.7
B 70	Deverra tortuosa (Desf.)DC. Syn. Pituranthus tortuosa (Desf.) Benth. ex Asch. & Schweinf.	15.7±0.58
B 71	Lavandula coronopifolia (L. stricta)	90.8±1.71
B 72	Echinops spinosissimus Turra	18.3±0.47
B 73	Echiochilon fruticosum Desf.	88.3±0.82
B 74	Alstona scholaris (L.)	65.9±0.62
B 75	Agava angustifolia Haw. Cv. Marginata	7.5±0.0
B 76	Bougainvillea spectabilis Willd.	4.3±0.76
B 77	Aloe arborescens Mill	12.1±1.8

The involvement of free radicals appears to be a feature of most, if not all human diseases, including cardiovascular disease and cancer (Fatma *et al*., 2000), therefore the activity of *Lycium europium* L., *Liomoniastrum monopetalum* (L.) Boiss., *Varthemia candicans* (Delile) Boiss. Jasonia candicans (Delile) Botsch., *Phlomis floccosa* D. Don, *Lavandula coronopifolia* Poir., *Thuja orientalis* and *Areca catechu* extracts is may be particularly important in fighting these diseases by conferring protection against free radical damage cellular DNA, lipid and proteins.

Total Antioxidant Activity (TAA):

The ABTS/H_2O_2/HRP discoloration method is reported to represent the total antioxidant activity of plant methanolic extract (Hsu *et al*., 2003). The total antioxidant activity of *Lycium europium* L., *Liomoniastrum monopetalum* (L.) Boiss., *Varthemia candicans* (Delile) Boiss. Jasonia candicans (Delile) Botsch., *Phlomis floccosa* D. Don, *Lavandula coronopifolia* Poir, *Thuja orientalis* and *Areca catechu* extracts is presented in Fig.1. The statistical analysis of TAA% data showed that the total antioxidant activity of all tested extracts significantly increased concentration dependently except *Lycium europium* L. which decreased concentration dependently. *Thuja orientalis*, *Areca catechu* and *Phlomis floccosa* D. Don represented the same activity level at 75µg/ml which were 18% over than standard antioxidant (L-ascorbic acid) at the same concentration.

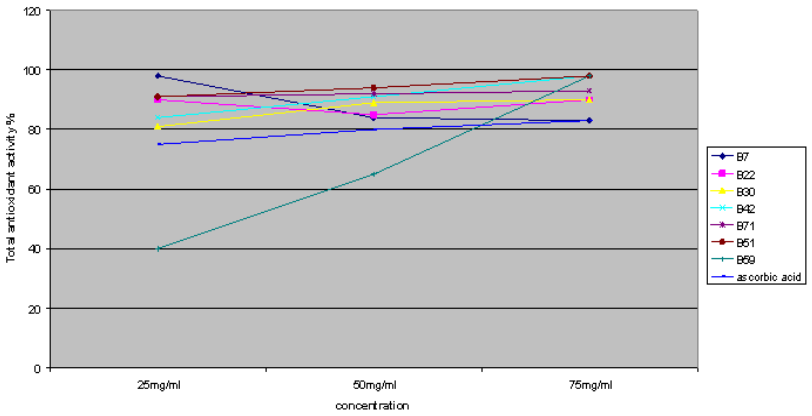

Fig. 1: Total antioxidant activity different extracts at different concentrations and ascorbic acid in peroxidase method, results are average of triplicat.

Lavandula coronopifolia Poir. had no significant difference between different concentrations also there is no significant difference between medium and highest concentration of *Lycium europium* L., *Liomoniastrum monopetalum* L., *Varthemia candicans* Delile. The recommended conc. for the highest antioxidant activity is 75µg/ml for all extracts except *Lycium europium* L. which was at 25µg/ml.

Ferric Reducing Antioxidant Power:

The reducing power has been used as one of the antioxidant capability indicators of medicinal herbs (Duh *et al.*, 1999). Fig. (2) Shows the reducing power of the extracts by using the potassium hexacyanoferrate reduction method compared to ascorbic acid and BHT.

The reducing power of all tested extracts showed the same trend of results of total antioxidant activity, it was increased concentration dependently. All concentrations of all extracts showed higher activities than the control in a statistically significant ($P < 0.05$) manner. Results presented in Fig. (2) shows that *Liomoniastrum monopetalum* L. extract has the lowest activity as compared to other extracts or standards while *Phlomis floccosa* D. Don and *Lavandula coronopifolia* Poir, *Thuja orientalis* and *Areca catechu* showed higher activities than those of ascorbic acid or BHT at 50µg/ml and 75µg/ml. The highest recorded reducing power activity is for *Thuja orientalis* at 75µg/ml, it represents 28& 33% increments over than ascorbic acid and BHT, respectively.

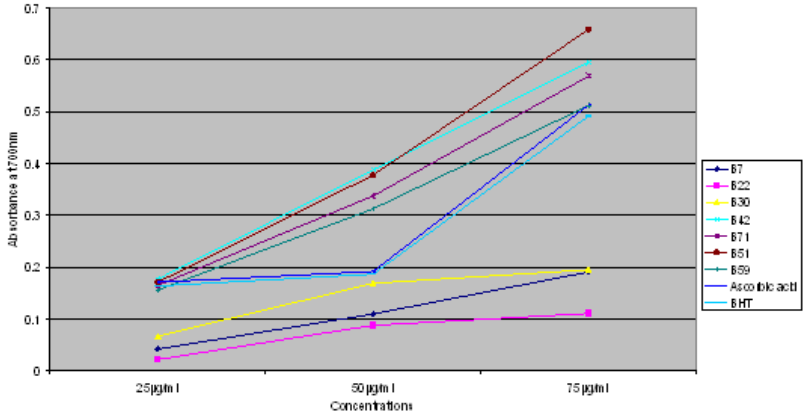

Fig. 2: Reducing power of different extracts at different concentration using potassium ferricyanide-ferric chloride method, values are mean of triplicates.

This effect may be due to the presence of polyphenolic constituent in genus: *Liomoniastrum* (Chaabi *et al*., 2008), genus *Lavandula* (Ulubelena *et al*., 2007), *Thuja orientalis* (Jung *et al*., 2010) and *Areca catechu* (wetwitayaklung *et al*., 2006).

Superoxide Anion Scavenging Activity:

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 (Oktay *et al*., 2003).

Data presented in Table, 3 show the inhibition percentage of superoxide radical generation by 25µg/ml, 50µg/ml and 75µg/ml of *Lycium europium* L., *Liomoniastrum monopetalum* (L.), *Varthemia candicans* (Delile)., *Phlomis floccosa* D., *Lavandula coronopifolia* Poir., *Thuja orientalis* and *Areca catechu* comparable to the same doses of ascorbic acid and BHT. The extracts of *Thuja* and *Areca* plants exhibited higher superoxide radical scavenging activity than ascorbic acid and BHT ($P < 0.05$).

Table 3: Superoxide scavenging activity of plant extracts at different concentrations.

Plant extracts	Inhibition percentage (%) (mean ± S.D.)		
	25µg/ml	50µg/ml	75µg/ml
Lycium europium L.	73±2.66	88.8±3.2	93.8±2.58
Liomoniastrum monopetalum (L.)	71±3.19	87.6±2.15	93.54±1.92
Varthemia candicans	71±5.06	87.2±1.63	93.4±1.44
Phlomis floccosa	76±2.18	89.2±3.07	93.8±1.62
Lavandula coronopifolia	70±1.25	86.8±3.67	90.6±2.9
Thuja orientalis L.	71±4.52	88.8±1.44	95.2±2.34
Areca catechu L.	71±2.42	90.36±2.82	95±2.41
Ascorbic acid	20±1.45	53.33±2.79	71.67±2.18
BHT	73.33±2.61	74.07±2.85	76.42±1.12

The percentage inhibition of superoxide generation by the maximum concentration of different extracts was found as the same for *Lycium*, *Liomoniastrum*, *Varthemi*a and *Phlomis* (93%) and they are greater than that of concentrations of ascorbic acid and BHT (71.67& 76.42%), respectively. The highest scavenging activity was recorded with Thuja and Areca plant extracts (95%) at 75µg/ml while the lowest activities were recorded for all extracts at the lowest tested concn.. Superoxide radical scavenging activity of examined samples at different concns. had the following order: *Areca* > *Thuja* > *Phlomis* > *Liomoniastrum* > *Varthemia* > *Lycium* > *Lavandula*.

Metal Chelating Activity:

The chelating of ferrous ions by the examined extracts was estimated using the method of Dinis *et al*. (1994). Ferrozine can quantitatively form complexes with Fe2+. In the presence of chelating agents, the complex formation is disrupted and eventually that the red color of the complex fades. Measurement of color reduction therefore allows estimation of the chelating activity of the co-existing chelator (Yamaguchi *et al*., 2000). In this study, B22 extract showed the highest activity in ferrous chelation as compared to other extracts or standards also it is greater than ascorbic acid and BHT by 28.3 and 38.3%, respectively. Metal chelation was increased concentration dependently with all plant extracts as mentioned in Fig. (3) and the activities of different extracts were as follows, *Liomoniastrum* > *Phlomis* > *Thuja* > *Varthemia* > *Areca* > *Lycium* > *Lavandula*.

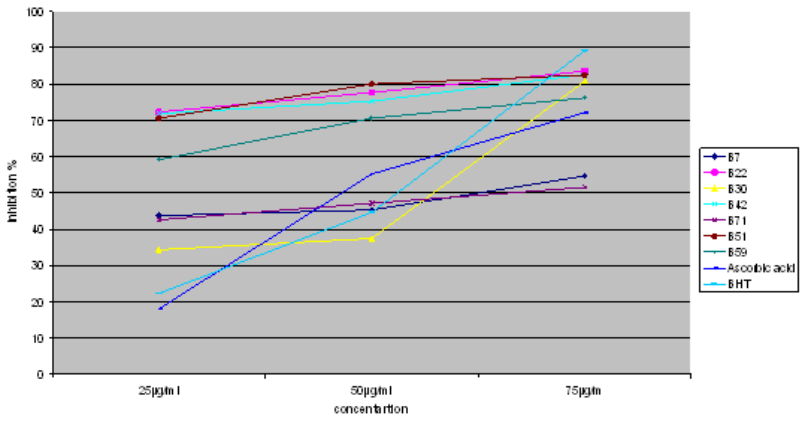

Fig. 3: Antioxidant activity measured by ferrous ion chelating assay

Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Chang *et al*., 2002; Halliwell, 1991). Metal chelating capacity is important since it reduced the concentration of the catalysing transition metal in lipid peroxidation (Duh *et al*., 1999). It was reported that chelating agents, that 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 (Gordon, 1990).

So tested extracts may be prevent lipid peroxidation by reducing the catal using transition metal and they are arranged as following *Liomoniastrum*, *Phlomis*, *Thuja*, *Varthemia*, *Areca*, *Lycium* and *Lavandula*.

Scavenging of Hydrogen Peroxide:

Hydrogen peroxide itself is not very reactive but it may be toxic to cell due to increase in hydroxyl radical concentration in the cell (Halliwell, 1991). Thus removal of H_2O_2 as well as O_2^- leads to increase survival of the cell and protect its components. The scavenging ability of examined extracts on hydrogen peroxides is comparable with that of standard L-ascorbic acid. All extracts at different concentrations exhibited scavenging activity against hydrogen peroxide in a dose dependant manner as shown in Fig.(4).

Lycium had potent scavenging activity against hydrogen peroxide, they showed the lowest absorbance of hydrogen peroxide followed by other extracts as following, *Liomoniastrum*, *Varthemia*, *Phlomis*, *Lavandula*, *Thuja*, *Areca*, ascorbic acid then BHT. The H_2O_2 scavenging capacity of extracts may be attributed to the structural features of their active components. According to the recent reports, a highly positive relationship between total phenols and antioxidant activity was found in many plant species (Vinson *et al*., 1998;Velioglu *et al*., 1998; Gülçin *et al*., 2002& Oktay *et al*., 2003).

So the antioxidant activity of tested extracts may be attributed to phenolic compounds content (Najla *et al*.(2009)& Ilef Limem-Ben Amor *et al*.(2009) which could donate electrons to H_2O_2, thus neutralizing it to water. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano *et al*., 1989).

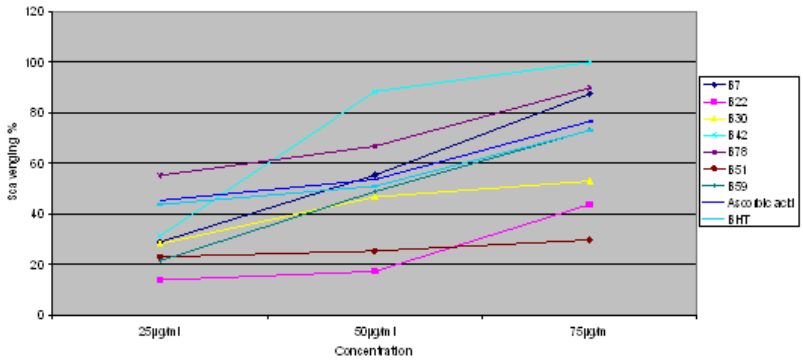

Fig. 4: Hydrogen peroxides scavening activity of selected plant extracts, Values are mean of three replicates.

Stated results are in coincided with those of Najla (2009) who reported that leaf extract of *Limoniastrum monopetalum* using pure methanol showed the highest polyphenol content (15.85mg as gallic acid/g extract& 4.2mg flavonoids as catechin/ g extract) and it showed a high DPPH radical scavenging, superoxide anion radical-scavenging and reducing power activities also Ilef Limem-Ben Amor *et al*. (2009) who stated that *Phlomis* has antioxidant properties due to its essential oil which some of them may be constituted in the methanolic extract. Flavonoids and iridoids prevented bleomycin-Fe (II) catalyzed arachidonic acid superoxidation (Couladis *et al*., 2003) that means powerful antioxidant activity also this plant was found to be potent scavengers of 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical (K"http://www.sciencedirect.com/science_ob=ArticleURL&_udi=B6T8D-4WMDHJY-1&_user=1966284&_coverDate=09%2F07%2F2009&_rdoc=1&_fmt=high&_orig=search&_origin=search&_sort=d&_docanchor=&view=c&_searchStrId=1521011150&_rerunOrigin=google&_acct=C000055643&_version=1&_urlVersion=0&_userid=1966284&md5=8e9c504c8b027ce42c18987af5a10153&searchtype=a"\l"bib19" Delazar *et al*., 2008).

Cytotoxic Activity of Selected Extracts on Different Cell Lines:

The MTT is well established method used to assess mitochondrial competence (Freshney, 2000). The seven methanolic extracts had no activity on HCT116 cell line, while methanolic extract of *Lavandula coronopifolia* and *Thuja orientalis L.* showed moderate activity against HEPG2 (46& 47.2%, respectively) as mentioned in Table 4.

Table 4: Cytotoxic effect of selected extracts on different carcinoma cell lines (HePG2, MCF7, HCT116, A549).

Plant name	Cytotoxicity (%) at 100 µg/ml			
	HEPG2	MCF7	HCT116	A549
Lycium europium L.,	0	63.2	0	54.8
Liomoniastrum monopetalum (L.) Boiss.,	16.7	43.4	0	25.3
Varthemia candicans (Delile) Boiss. Jasonia candicans (Delile) Botsch.,	37.5	0	0	12.5
Phlomis floccosa D. Don,	0	9.2	4.6	42.5
Lavandula coronopifolia Poir.	46	19.5	2.5	13.7
Thuja orientalis L.	47.2	0	0	22.9
Areca cttechu L.	0	48.3	0	0

The methanolic extract of *Lycium europium* L., *Liomoniastrum monopetalum* (L.) and *Areca catechu* L. gave moderate activity on MCF7 cell line (63.2 & 48.3%, respectively). In case of A549 cell line, *Lycium europium* L. and *Phlomis floccosa* D. Don showed the best results (54.8& 42.5%, respectively).

Conclusion:

The results obtained using five different methods to evaluate the antioxidant activity (DPPH, FRAP, TBARS, FIC, superoxide scavenging and hydrogen peroxide scavenging) showed that *Thuja orientalis* and *Areca catechu* L. are the most effective antioxidant and they can be considered as good sources of natural compounds with significant antioxidant activity, which can be attributed to the high percentage of polyphenolic compounds content. The seven antioxidant plant extracts didn't show detectable cytotoxicity at the applied concentration on HCT116 cell lines while they have different effects on HEPG2, MCF7 and A549 cell lines

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