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Medicinal plants possessed antioxidant and free radical scavenging effects (part 3)- A review

Prof Dr Ali Esmail Al-Snafi
Department of Pharmacology, College of Medicine, Thi qar University, Iraq

Abstract:- Antioxidants are substances that remove, prevent or delay oxidative damage to a target molecule. Therefore, an antioxidant may act to control the level of free radicals to counteract oxidative damage. The effects of medicinal plants in prevention and treatment of many diseases have been widely attributed to their antioxidants activities. This review was designed to highlight the antioxidant effects and free radical scavenging activity of medicinal plants as a third part of our previous reviews.

Keywords*: antioxidant, free radical scavenging, medicinal plant, herbs*

I. INTRODUCTION:

Previous reviews showed that a wide range of medicinal plants exerted antioxidant and free radical scavenging activity [1-8], including *Achillea santolina* [9], *Adiantum capillus-veneris* [10], *Agrimonia eupatoria* [11], *Ailanthus altissima* [50], *Alhagi maurorum* [12], *Allium species* [13], *Alpinia galangal* [14], *Althaea officinalis* [15], *Ammannia baccifera* [16], *Ammi visnaga* [17], *Anchusa italica* [18], *Anchusa strigosa* [19], *Anethum graveolens* [20], *Antemis nobilis* [21], *Antirrhinum majus* [22], *Arachis hypogaea* [23], *Arctium lappa* [24], *Artemisia campestris* [25], *Asparagus officinalis* [26], *Astragalus hamosus* [27], *Avena sativa* [28], *Bacopa monniera* [29], *Ballota nigra* [30], *Bauhinia variegata* [31], *Bellis perenni* [32], *Bidens tripartite* [33], *Brassica rapa* [34], *Bryophyllum calycinum* [35], *Caesalpinia crista* [36], *Calamintha graveolens* [37], *Calendula officinalis* [38], *Calotropis procera* [39], *Canna indica* [40], *Capparis spinosa* [41], *Capsicum specie* [42], *Carthamus tinctorius* [43], *Carum carvi* [44], *Cassia occidentalis* [45], *Casuarina equisetifolia* [46], *Centaurea cyanus* [47], *Chenopodium album*[48], *Chrozophora tinctoria* [49], *Cicer arietinum* [50], *Cihorium intybus* [51], Citrus species [52], *Clerodendrum inerme* [53], *Clitoria ternatea* [54], *Colchicum balansae* [55], *Convolvulus arvensis* [56], *Coriandrum sativum* [57], *Coronilla scorpioides* [58], *Cotoneaster racemiflora* [59], *Cressa cretica* [60], *Crocus sativus* [61], *Crotalaria juncea* [62], *Cuminum cyminum*[63], *Cupressus sempervirens* [64], *Cydonia oblonga* [65], *Cynodon dactylon* [66], and *Cyperus rotuntdus* [67]. This review was designed to highlight the antioxidant effects of medicinal plants as a third part of our previous reviews.

Medicinal plants possessed antioxidant and free radical scavenging activity:

Dactyloctenium aegyptium

The radical scavenging activity of the crude extract of *Dactyloctenium aegyptium* was determined by DPPH method. *Dactyloctenium aegyptium* crude extract had showed high percent radical scavenging activity (66.59), but less when compare to standard, ascorbic acid (78.40) [68-69].

Dalbergia sissoo

Extract of bark of *Dalbergia sissoo* Roxb (Fabaceae) was assessed for its antioxidant activity by in vitro methods. Antioxidant activity was studied using hydrogen peroxide scavenging activity and reducing power assay. The extracts exhibited significant antioxidant activity [70]. The ethanol extract of the bark of *D. sissoo* was screened for antioxidant potential. Lipid peroxidation inhibitory (LPO) and NO quenching potentials of *D. sissoo* were investigated. The bark extract showed 69.1% LPO inhibitory potential/10 µg of extract, and trolox was used as positive control. Superoxide dismutase (SOD) mimetic activity was 116.62 unit/min/mg [71]. The antioxidant activity of the aqueous extract and methanol extracts of the stem bark of the plant, *Dalbergia sissoo* was evaluated by in vitro chemical analyses involving the assays of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, ferric ion reducing power, ferrous ion chelating activity and and Au nanoparticle formation potential. In all the assays, aqueous extract showed significantly greater activity than methanol extracts [72]. The in vitro antioxidant activity of the successive petroleum ether (PEDS), chloroform (CEDS) and methanol (MEDS) extracts of the stem bark of *Dalbergia sissoo* was investigated through DPPH free radical scavenging activity, reducing power, FRAP (ferric reducing antioxidant power) assay, ferrous ion scavenging activity and nitric oxide (NO) radical scavenging activity. Among different extracts, the chloroform

extract was found to be most potent showing the IC50 of 25 µg/ml for DPPH model, 21µg/ml for reducing power, 26 µg/ml for ferrous ion scavenging, 26 µg/ml for FRAP assay, 25 µg/ml for NO scavenging activity, which were comparable to positive control (ascorbic acid). The activity of petroleum ether and methanol extract was found to be moderate. Total phenolic contents of the various extracts were estimated as 50.8 mg/g. Strong positive correlation was recorded between the antioxidant activity and total phenolic content of the different extracts [73]. Antioxidant activity of methanolic extract of *Dalbergia sissoo* root was investigated for its free radical scavenging activity by determining the nitric oxide and hydrogen peroxide scavenging activity. Maximum scavenging of nitric oxide and hydrogen peroxide found were 26.66% and 50.68% respectively at 250 µg/ml concentration. The results were compared with rutin as a standard [74]. Three water-soluble polysaccharides were isolated and purified from the leaves of *Dalbergia sissoo* Roxb. (DSLP), bark. Antioxidant and moisture preserving activities of these three polysaccharides were investigated using in vitro methods. The antioxidant activities studied include superoxide O^{2-}, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, hydroxyl OH^{-}, nitric oxide (NO), N,N-dimethyl-p-phenylenediamine (DMPD) radical scavenging activities, ferric ion Fe^{3+} reducing ability, ferrous ion (Fe^{2+}) chelating and lipid peroxidation activities. The result revealed DSLP possessed antioxidant characteristics. Furthermore, the three polysaccharides showed effective moisture retention properties in comparison with hyaluronic acid and glycerol [75].
4,2′,5′-trihydroxy-4′-methoxychalcone, (2*S*)-6,4′- dihydroxy-7-methoxyflavan, 6,4′-dihydroxy-7-methoxyflavanone, *R*(+)-4-methoxydalbergione, *R*(-)-latifolin, *R*(+)-dalbergiphenol, 9-hydroxy-6,7-dimethoxydalbergiquinol and isoparvifuran isolated from the heartwood of *D. odorifera* showed a protective effect on glutamate-induced oxidative injury in HT22 cells. All compounds were tested at the concentrations of 1, 10, 20 and 50 µM and compared with trolox (positive control). EC_{50} values were of 7.47, 2.85, 3.3, 8.54, 5.82, 6.54, 8.14 and 3.09 µM, for these compounds respectively in comparison with trolox which showed EC50 value of 15.8 µM [76-77].

Daphne mucronata

The antioxidant activity of eleven compounds isolated from *Daphne mucronata* was studied using DPPH assay. Compound 5,7,3′,4′-tetrahydroxyflavone and 5,3′,4′- trihydroxyflavone 7-O-ß-D-glucopyranoside showed moderate antioxidant activity while, other compounds including (cinnamic acid, 7,8-dimethoxycoumarin, 7,8-dihydroxycoumarin, lupeol, ßamyrin, betulin, 5,6,7,8,3′,4′-hexamethoxyflavone, 5-hydroxy-3,6,7, 4′tetramethoxyflavone, and stigmasterol 3-O-ß-D-glucopyranoside) were weak antioxidants [78-79].

***Datura fastuosa* (syn: *Datura metel*)**

Hydro alcoholic and methanolic seed extracts of *D. fastuosa* were evaluated for antioxidant potential, total antioxidant capacity, total amount of phenolic content, total flavonoid content, total flavonols and total proanthocyanidines content. The IC_{50} value using DPPH model for methanolic extract of *D. fastuosa* was 28.34 µg/ml & for hydroalcoholic extract 25.78 µg/ml. The values of total antioxidant capacity, total amount of phenolic content, total flavonoid content, total flavonols and total proanthocyanidines content for methanolic extract of *D. fastuosa* were found to be 6.83 mg/g, 9.97 mg/g, 6.34 mg/g, 5.37 mg/g and 1.42 mg/g of plant extract respectively and for hydroalcoholic extract, the values for the same parameters were 7.44 mg/g, 6.88 mg/g, 9.35 mg/g, 5.36 mg/g and 0.88 mg/g respectively [80]. The antioxidant activity of different solvent extracts from the leaves of *Datura metel* was tested by DPPH scavenging activity, hydroxyl radical scavenging activity, reducing power assay, and β –carotene bleaching activity. The antioxidant activity was performed at four concentrations ranging from 25-100 mg/ml. Chloroform extract exhibited highest concentration dependent antioxidant activity [81]. In vitro antioxidant activity of the extract was performed by 2, 2-diphenyl-1-picrylhydrazyl radical scavenging method. A positive correlation was recorded between the phenolic and flavanoid content of the *Datura metel* extracts with the free radical scavenging activities [82-83].

Datura stramonium

The antioxidant activity of the plant extracts was assessed on the basis of the free radical scavenging effect on the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) by modified method. *D. stramonium* leaf extracts exhibited potent antioxidant property [84].

Daucus carota

The effects of a 3-week supplementation of the diet with carrot (15% dry matter) in antioxidant status was studied in rats. Carrot consumption improved the antioxidant status. It significantly decreased the urinary excretion of thiobarbituric acid reactive substances (TBARS), reduced the TBARS levels in heart, increased the vitamin E plasmatic level and tended to increase the ferric reducing ability of plasma (FRAP) as compared to

the controls. The carrot diet provided carotenoid antioxidants: 5.1 mg β-carotene, 1.6 mg α-carotene and 0.25mg lutein per 100 g diet. No carotenoids were found in plasma whereas the three carotenoids were detected in the plasma of the rats fed the carrot diet at 125, 41, 43 nmol/l respective concentrations. β-Carotene was also detected in liver and heart [85-86].Antioxidants and antioxidant capacity of seven colored carrots were studied. Antioxidant capacities of the hydrophilic and hydrophobic fractions were determined using the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) methods. Five anthocyanins, chlorogenic acid, caffeic acid, and four carotenoids were quantified by HPLC. Anthocyanins were the major antioxidants in purple-yellow and purple-orange carrots, and chlorogenic acid was a major antioxidant in all carrots. Carotenoids did not contribute to total antioxidant capacity, but correlated with antioxidant capacity of hydrophobic extracts. Both the DPPH and ABTS assays showed that the hydrophilic extract had higher antioxidant capacity than the hydrophobic extract. Purple-yellow carrots had the highest antioxidant capacity, followed by purple-orange carrots, and the other carrots did not significantly differ [87].
The major constituents isolated from the methanol extract of *D. carota* seeds were luteolin, luteolin 3'-O-beta-D-glucopyranoside and luteolin 4'-O-beta-D-glucopyranoside were studied for antioxidant effects. Among these three flavones, luteolin showed the highest degree of free radical scavenging activity ($RC50 = 4.3 \times 10^{-4}$ mg/ml) in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [88]. Purple carrot (PC) extract subjected to digestion process. Results indicated that the extract obtained upon passage through the gastrointestinal tract, which came into contact with the colonic cells in situ, was less potent than the extract, which was not subjected to digestion process. Digested PC extract exhibited intracellular ROS-inhibitory capacity, with 1mg/ml showing the ROS clearance of 18.4%. A 20.7% reduction in oxidative DNA damage due to colon mucosa cells' treatment with digested PC extract was observed [89]. The in vitro antioxidant activity of *Daucus carota* oil extract (DCOE) was evaluated using 1,1-diphenyl-2-picryl hydrazyl free radical scavenging assay (DPPH), ferrous ion chelating assay (FIC) and the ferric reducing antioxidant power assay (FRAP). DCOE exhibited antioxidant activity in all assays used. The FRAP value was 164 ± 5.5 µmol $FeSO_4$/g, and the IC_{50} values for DPPH and FIC assays were 2.1 ± 0.03 mg/ml and 0.43 ± 0.02 mg/ml, respectively [90].The antioxidant activity of the essential oils from the fruits of *Daucus carota* var. sativus (yellow carrot) and var. boissieri (red carrot) was investigated using DPPH•.Both oils were able to reduce DPPH• and to prevent the degradation of the deoxyribose sugar in a concentration dependent manner. Carrot oils showed promising scavenging activity of DPPH• with an IC_{50} of 12.71 mg/ml and 14.15 mg/ml for the yellow and red carrot oils, respectively [91].
The antioxidant activity of wild *Daucus carota* extracts seed (70% and 40% ethanol) was investigated. The results indicated that the 70% ethanolic extract has a higher total phenol and flavonoid content than 40% ethanolic extract. The antioxidant activity of 70% ethanolic extract was 86.88 % ± 3.018, whereas of 40% ethanolic extract scavenging activity was 78.72% ± 3.276 [92].

The n-Hexane, ethyl acetate, and methanol extracts of leaves of *D. carota* were examined for free radical scavenger activity using DPPH method. The compounds possessed antioxidant activity in the n-hexane extract was lutein. The compound at concentration of 0.616, 1.025, and 2.05 ppm had antioxidant activities of 0.94 ± 0.05; 18.53 ± 0.15; and 49.07 ± 0.86, respectively [93].

The antioxidant features of the black carrot extracts were shown to be significantly higher than those of orange carrots, the recorded reducing capacity of the two black carrots extracts was (86.4 ± 8.0 and 182.0 ± 27 µM TE/100 g fw) and the radical scavenging ability was (17.6 ± 9.0 and 240.0 ± 54.0 µM TE/100 g fw) [94].
High-carbohydrate, high-fat diet-fed rats developed hypertension, cardiac fibrosis, increased cardiac stiffness, endothelial dysfunction, impaired glucose tolerance, increased abdominal fat deposition, altered plasma lipid profile, liver fibrosis and increased plasma liver enzymes together with increased plasma markers of oxidative stress and inflammation as well as increased inflammatory cell infiltration. Purple carrot juice reversed all these parameters. Anthocyanins were responsible for the antioxidant and anti-inflammatory properties of purple carrot juice and improvement of glucose tolerance and maintaining cardiovascular and hepatic structure and function, while, β-carotene did not reduce oxidative stress, cardiac stiffness or hepatic fat deposition [95].

Desmostachya bipinnata

The antioxidant activities of different concentrations of 70% methanolic extract of the roots of *Desmostachya bipinnata* were examined for different reactive oxygen species (ROS) scavenging activities including DPPH, nitric oxide, hydrogen peroxide and hydroxyl radical scavenging activities. The extract has shown effective antioxidant activity in all assay techniques. Furthermore, *Desmostachya bipinnata* was found to be most potent scavenger of hydrogen peroxide with IC_{50} value 127.07± 6.44 µg/ml against standard ascorbic acid 122.60± 2.14 µg/ml [96-97].The antioxidant and DNA damage protection activity of hydroalcoholic extract of *Desmostachya bipinnata* was investigated in both in vitro and in vivo studies. The extract showed significant antioxidant activity in a dose-dependent manner with an IC_{50} value of 264.18 ± 3.47 µg/ml in H_2O_2 scavenging assay and prevented the oxidative damage to DNA in presence of DNA damaging agent (Fenton's reagent) at a concentration of 50 µg/ml. Also, the presence of extract protected yeast

cells in a dose-dependent manner against DNA damaging agent (Hydroxyurea) in spot assay. Moreover, the presence of extract exhibited significant antioxidant activity in vivo by protecting yeast cells against oxidative stressing agent (H_2O_2) [98-99].

Dianthus caryophyllus

The scavenging effect of volatile oil of *Dianthus caryophyllus* flowers was studied using DPPH assay. The plant possessed scavenging effect, but wen it was treated with 400 ppm stigmasterol, it gave the highest scavenging activity [100-101].

Digitalis purpurea

The scavenging activity of alcoholic extract of *D. purpurea* was measured using DPPH and the total antioxidant capacity of *D. purpurea* was measured by phosphomolybdate using ascorbic acid as the standard. *D. purpurea* 1mg/ml showed 94.25% DPPH scavenging activity and 92.28% total anti-oxidant activity [102-103].

Dodonaea viscosa

Dodonaea viscosa started to exhibit a high effective free radical scavenging at 50 µg/ml (50.72%) and it was concentration dependently increased reaching 92.45% at 1000 µg/ml. The water extract was only weak antioxidant. Water extract free radical scavenging reached 31.80% at the highest concentration 1000 µg/ml [104].100, 200 and 300 µl of *Dodonaea viscosa* extract showed that a maximum scavenging activity was offered by 300 µl of *Dodonaea viscosa* extract with inhibition of 82.09 ± 0.15%, followed by 200 µl with inhibition of 81.02 ± 0.11%, and 100 µl with inhibition of 79.91 ± 0.16% [105-106].

Dolichos lablab

The antioxidant effect of methanol extracts of two Bangladeshi bean pods namely *Lablab purpureus* L. sweet white and purple was studied using DPPH free radical scavenging method. In DPPH test the lowest and highest IC_{50} values were 430.00µg/ml and 853.13µg/ml, with *L. purpureus* sweet purple and *L. purpureus* sweet white respectively. The total Flavonoid contents of the test samples are 42.55±5.77 and 32.09±0.36 mg/g quercetin equivalents for 'white' and 'purple' respectively [107-108].

Echinochloa crus-galli

The results of scavenging activity indicated that all seeds extracts showed excellent scavenging activities in all tests at a concentration range from 10-50 µg /ml in the reaction mixture and increased steadily with the increased concentration. IC_{50} for scavenging effects of 1% acidified methanol extract of *Echinochloa crus-galli* were DPPH scavenging activity 686.0497±8.0304 µg/ml, β-carotene bleaching effect 140.165±2.49 µg/ml and H_2O_2 26.947±0.55 µg/ml. However, IC_{50} of 95% ethanol extracs of *Echinochloa crus-galli* were DPPH scavenging activity 226.4637±13.086 µg/ml, β-carotene bleaching effect 143.656±1.96 µg/ml and H_2O_2 21.5875±0.52 µg/ml, while, IC_{50} of aqueous extract of *Echinochloa crus-galli* were DPPH scavenging activity 199.5967±8.2838 µg/ml, β-carotene bleaching effect 130.67±4.81 µg/ml and H_2O_2 39.624±1.542 µg/ml [109].Antioxidant property of *Echinochloa crus-galli* aerial parts extracts was investigated in vitro. IC_{50} for various extracts were determined for DPPH scavenging, bleaching of β- carotene and % inhibition of H_2O_2 and compared with standard positive controls viz. butylated hydroxytoluene (BHT) for DPPH, propyl gallate for β-carotene and ascorbic acid for H_2O_2 assay. All extracts (1% Methanol, 95% Ethanol and aqueous) exerted antioxidant activity by all antioxidant tests utilized in the study [110-111].

Echium italicum

The antioxidant effect of the ethanol extracts from the roots and herbs of *Echium italicum* L. E. vulgare L was investigated by DPPH free-radical scavenging, Fe^{2+}- chelating ability, total phenolic contents and total flavonoid contents methods. Extracts showed a concentration-response relationship in DPPH scavenging activity. An increase in the concentration is synonymous with an increase in scavenging capacity. But root extracts of the showed more potent DPPH scavenging activity and Fe^{2+}- chelating ability than the herb extracts. DPPH free radical scavenging activities % for 50, 100, 500 and 1000 µg/ml of herb ethanolic extract were 3.18 ± 0.02, 16.49 ± 0.01, 25.90 ± 0.06 and 33.44 ± 0.03, and of root ethanolic extract were 11.93± 0.01, 13.83 ± 0.02, 57.24 ± 0.01 and 81.43 ± 0.01 respectively. The ferrous Ion chelating activities % of the ethanol extracts for 200 and 400 µg/ml of herb ethanolic extract were 5.93 ± 0.04 and 7.26 ± 0.06 , and of root ethanolic extract were 25.93 ± 0.02 and 32.0 ± 0.06 respectively [112-113].

Ephedra alata

The antioxidant activity of *Ephedra alata* was evaluated by using 2, 2-diphenyl-1-picryl-hydrazyl-hydrate assay. The *E. alata* methanolic extract showed high antioxidant activity and powerful oxygen free radical scavenging abilities as well as the IC_{50} for the plant was almost equivalent to the Trolox standard antioxidant [114-115].

Equisetum arvense

The plant contained high amount of polyphenols. Antioxidant activity (ABTS assay) was estimated to be 98.13± 3.84 (μM Trolox equivalents/g dry weight) [116-117]. The total phenol content, total antioxidant capacity and silicic acid amount were found to be 18.67 %, 123 mg gallic acid/g dry weight extract, 1608 μM TEAC/mg dry weight extract and 0.0049 mg silicic acid/mg dry weight extract, respectively [118].Aqueous and ethanol extract from top and body portions of field horsetail (tsukushi) were tested for antioxidative activity using four different methods. The ethanol extract fractions of each portion were richer in total phenolic components than water extracts. These fractions had remarkable antioxidative activities, similar to that of 5 mM ascorbic acid. Water extracts of both portions showed high superoxide anion radical-scavenging activities. Hydroxyl radicals were effectively scavenged by ethanol extracts. Field horsetail (tsukushi) was rich in vitamins C and E. and contained high levels of copper and zinc. These were essential elements, for superoxide dismutase to act against active oxygen species [119].

The antioxidative activity of different horsetail (*Equisetum arvense* L.) extracts was studied by the electron spin resonance (ESR) spectroscopy–spin trapping method. The influence of different horsetail extracts during lipid peroxidation of sunflower oil induced by the lipophilic azo-initiator 4,4'-azobis(4-cyanovaleric acid) and soybean phosphatidylcholine liposomes induced by the hydrophilic azo-initiator 2,2'-azobis(2-amidinopropane) dihydrochloride was investigated. The results of ESR analysis confirmed that the extracts investigated suppressed the formation of lipid peroxyl radicals in both systems investigated in a dose-dependent manner. The results indicate that n-butanol, methanol, ethyl acetate, and water extracts had significant peroxyl radical scavenging activity [120].

The antioxidant activity and phenolic composition of three different extracts (Ethanol, n-Butanol and H_2O) of field horsetail (*Equisetum arvense* L.) were investigated by measuring the total reducing power (expressed by Ascorbate Equivalent Antioxidant Capacity - AEAC), inhibition of lipid peroxidation, and free radical scavenging capacity (RSC) towards 2,2-diphenyl-1- picrylhydrazyl (DPPH radical) and nitric oxide (NO), respectively. The results showed that the highest RSC regarding both DPPH and NO radicals was expressed by Ethanol extract (EC_{50}=2.37 μg/ml and EC_{50}=90.07 μg/ml, respectively), and the lowest by H_2O extract (EC_{50}=37.2 μg/ml and EC_{50}>333.33 μg/ml, respectively), while, n-Butanol extract showed the highest total reducing power (AEAC=13.40 μg/ml) [121].

The effect of *Equisetum arvense* extracts as sources of natural antioxidants was studied. DPPH scavenging activity of *E. arvense* was 96.2% at 4.0 mg/ml foliage and central stalk, while, DPPH scavenging activity for rhizomatous stem and root was 94.7% at same concentration [122].

The antioxidative activity of horsetail extracts was tested by measuring their ability to scavenge stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reactive hydroxyl radicals by electron spin resonance spectroscopy. The results demonstrated that the free radical scavenging activity (versus both DPPH and hydroxyl radicals) depended on the type and concentration of applied extracts; the highest DPPH (EC_{50} = 0.65 mg/ ml) and hydroxyl radical scavenging activities (EC_{50} = 0.74 mg/ ml) were obtained in the case of *n*-butanol extract. The radical scavenging activity of extracts significantly correlated with total phenolic content [123]. Onitin and luteolin isolated from the methanolic extract of *Equisetum arvense* showed superoxide scavenging effects (IC_{50} = 35.3 ±0.2 microM and 5.9 ± 0.3 microM, respectively) and DPPH free radical scavenging effect (IC_{50} of 35.8 ±0.4 microM and 22.7 ±2.8 microM, respectively) [124].

***Erigeron canadensis* (syn. *Conyza canadensis*)**

The crude methanolic extract and different solvent fractions (hexane, chloroform, ethyl acetate and butanol) were tested for antioxidant activity using DPPH free radical activity. The maximum antioxidant potential at 100 μg/ml of ethyl acetate, aqueous fraction, *n*-hexane and chloroform fraction was 70.6, 71.65, 66.50 and 38.09 % with EC_{50} value 50.35, 46.34 and 44.55 μg/ml respectively [125-126].Treatment with 70% ethanolic extract of the aerial parts of *E. canadensis* decreased NO production in a murine macrophage cell line, Raw 264.7 in a dose-dependent manner as follows: 25, 40 and 64% reductions, respectively, at the concentrations of 1, 10 and 100 mg/ml. In addition, Extract did not affect on the cell viability and it showed potent DPPH radical scavenging activity [127].The antioxidant and protective effects of the plant extract were studied on plasma proteins against oxidative/nitrative damages induced by $ONOO^-$. Peroxynitrite evokes oxidative stress and induces undesirable effects in biological systems and causes damage to biomolecules. The extract (50 – 2500 mg/ml) caused a dose-dependent reduction of protein nitration by 90%. The oxidation of plasma proteins was diminished by about 75%. $ONOO^-$ oxidized the plasma thiol groups and this process was

inhibited by tested extract. The level of reduced protein thiols was increased thrice at the lowest concentration of extract (50 mg/ml). The highest concentration of extract decreased twice the level of protein thiols in reduced forms and increased the homocysteine level about 4.5 times. Accordingly, extract possessed antioxidative properties *in vitro*, protected plasma proteins against toxicity induced by peroxynitrite and had modulating effects on thiol/disulfide redox status [128].The protective effects of the polysaccharide extract from the plant on platelet proteins against nitrative and oxidative damage induced by $ONOO^-$ were studied . The extract of the plant distinctly reduced oxidation and nitration of proteins in blood platelets treated with $ONOO^-$ (0.1 mM) and O_2 production in these cells. . The ability of the extract to decrease O_2 generation in blood platelets supports the importance of free radicals in platelet functions, including aggregation process [129].

Erodium cicutarium

Extracts from *Erodium cicutarium* L. extracts were tested for their antioxidative properties using Fe^{2+}-induced triglyceride oxidation. Hydrophobic fractions such as petroleum ether, benzene and chloroform I extracts as well as hydrophilic fractions (water and ethyl acetate) possessed antioxidative effect. Tannin, catechins, gallic and elagic acids, sugars (glucose, galactose, fructose) amino acids (glycine, alanine, proline, histidine, tryptophan, tyrosine, glutamic acid), vitamins K and C were identified in *Erodium cicutarium* extracts. Standard samples of all these substances were tested for their antioxidative activity. Only polyphenolic compounds (tannin, gallic acid, (+)-catechin and vitamin C exhibited strong antioxidative properties [130-131]. Methanol extracts of nine species of Geraniaceae including *Erodium cicutarium* (L.) L'Hér. were studied for their antioxidant properties using DPPH (1,1-diphenyl-2-picryl hydrazyl) radical assay. Methanol extract of *Erodium cicutarium* exhibited considerable free radical scavenging activity and its IC_{50} values was below 50 µg/ml [132]. The methanol extract contained quercetin, kaempferol, myricetin, their mono- and di-glycosidic derivatives, and free polyphenolic acids. Low concentrations of the extract stimulated, and high concentrations inhibited, the free radical activity of human granulocytes in vitro [133-134].

Eryngium creticum

In estimation of antioxidant activity of *Eryngium creticum*, it appeared that the antioxidant activity of the aqueous extracts of the leaves and stems of *Eryngium creticum* was increased with increasing the concentration of the aqueous extract. This increase has reached 59 % and 35 % at the concentration 0.5 mg/ml for the leaves and stems respectively. Studies on the same plant but from an altitude 300 m showed that the leaves and stems at the same concentration (0.5 mg/ml), exerted an antioxidant activity of 90 % and 75 % respectively. On the other hand, at a concentration of 0.5 mg/ml, the ethanolic extract showed an important antioxidant activity of both leaves (81 %) and stems (79 %). However, this antioxidant capacity was highest for the plant that grows at 300 m. It has reached the 93 % for the leaves and 82 % for the stems. In addition, methanolic extract showed that both leaves and stems have exerted an important antioxidant activity reaching 90 % and 79 % respectively at a concentration of 0.5 mg/ml [135-136].

Antioxidant power of the Lebanese *E. creticum* was determined by DPPH assay using different extraction techniques. The results indicated that the antioxidant effect was increased with the concentration, the highest antioxidant activity in all the extraction techniques was obtained at a concentration of 0.5 mg/ ml [137-138]. The *in vitro* antioxidant activity of the aqueous and ethanolic extracts from different parts (leaves, stems, roots, and the whole plant) of the fresh plant *E. creticum* from the first and second harvest, were performed using antioxidant DPPH radical scavenging and superoxide radical scavenging. The results showed that the 4 parts of this plant have exerted antioxidant activity that may be due to their phenolic content. The antioxidant activity of the aqueous extract of leaves, stems and roots of *E. creticum* was increased with increasing concentrations. It reached 77% , 89% and 70% at the concentration of 0.5 mg/ml of leaves, stems and roots, respectively, for the first harvest, while, it reached 73%, 59% and 34% at the same concentration of the leaves, stems and roots respectively for the second harvest. The aqueous extract of the whole plant showed an antioxidant activity of 72% at the same concentration for the first harvest and 49% for the second. The ethanolic extract showed an antioxidant activity of 93%, 82% and 44% for leaves, stems and roots, respectively, at a concentration of 0.5 mg/ml for the first harvest whilst for the second harvest this activity was about 56%, 65% and 61% at the same concentration for the leaves, stems and roots, respectively [139].The antioxidant effect of three extracts (aqueous, methanolic and ethyl acetate) from fresh leaves and stems of *Eryngium creticum* was studied. The results showed that both leaves and stems extracts of the plant exerted an antioxidant activity that may be due to their phenolic content [140].The consumption of 100 g of fresh *E. creticum* leaves and stems provided antioxidants activity equivalent to (78.50±0.80) and (50.42±0.50) mg of vitamin C, respectively [141].

***Eucalyptus* species**

The effects of 1,8-cineole on systolic blood pressure (SBP) and oxidative stress was investigated in rats chronically exposed to nicotine. 0.1 mg/kg 1,8-cineole significantly reduced SBP, and 1.0 mg/kg 1,8-

cineole significantly increased plasma nitrite concentrations, compared with rats chronically exposed to nicotine alone. Rats chronically exposed to nicotine showed a significant increase in lipid peroxidation levels, an elevation significantly antagonized by treatment with 0.01 mg/kg and 0.1 mg/kg 1,8-cineole. Chronic exposure to nicotine also significantly increased plasma corticosterone levels, but this effect was not diminished by treatment with 1,8-cineole [142].The *in vitro* antioxidant activities of the essential oil and the subfractions of methanol extract from leaves of *Eucalyptus largiflorens* (*Eucalyptus bicolor*) were investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene-linoleic acid assays. In DPPH, the IC_{50} of polar subfractions of the methanol extract was lower than that of non polar one, while in the case of the linoleic acid system; oxidation of the linoleic acid was effectively inhibited by the non polar subfraction of the methanol extract (92 - 94.5 %), which was comparable to the synthetic antioxidant BHT. In both evaluating methods, the oils were less effective. The amounts of total phenolics in the polar subfractions of each extracts were positively correlated with the antioxidant activity [143].

The vitro antioxidant activity of the essential oil and methanol extracts of Eucalyptus largiflorens (*Eucalyptus bicolor*) was studied. The extract showed better antioxidant activity than the essential oil. Also, the polar subfraction of methanol extract showed the highest radical-scavenging activity. The inhibition capacity (%) of the nonpolar subfraction was found to be the stronger one [144]. The free radical scavenging activities of the *E. camaldulensis* essential oil were assessed by measuring their scavenging abilities for stable 2,21-diphenyl-1-picrylhydazyl DPPH radicals. The DPPH scavenging activity was high in E. camaldulensis (81.9%) [145]. The results of evaluation of antioxidant effect of essential oil of *Eucalyptus camaldulensis* indicated that the essential oil had weak radical scavenging activity comparing with ascorbic acid and Butylated hydroxyanisole (BHA), while it had high potent ferrous ions chelating and total antioxidant activities comparing to ascorbic acid and BHT [146]. The leaves extract of *Eucalvptus camaldulensis* var. brevirostris, grown in Nile delta in Egypt, were examined for the antioxidant activity. The extracts obtained by ethanol digestion and by supercritical fluid extraction showed the most promising antioxidative activities. In order to identify the most active compounds, the contents of both extracts were separated by reversed-phase HPLC. Gallic and ellagic acid were found to be the prevailing antioxidants in the ethanolic extract [147].

The antioxidant activity of the essential oils of *Eucalyptus camaldulensis* Dehnh, was assessed by DPPH-test and expressed as Trolox equivalent antioxidant capacity, they showed values ranging between 0.5 and 5.8 mmol/l [148].The antioxidant activities of *Eucalyptus camaldulensis*, *Eucalyptus camaldulensis* var. obtusa and *Eucalyptus gomphocephala* essential oils was studied (2,2'-diphenypicrylhydrazyl). The values of total antioxidant activity were 70 ± 3.13%, 50 ± 3.34% and 84 ± 4.64% for E. *camaldulensis, E. camaldulensis* var. obtusa and *E. gomphocephala*, respectively. The highest antioxidant activity value of 84 ± 4.64% could be attributed to the high amount of spathulenol (37.46%) [149].The effects of essential oil from *Eucalyptus camaldulensis* flowers oil on melanogenesis and the oil's antioxidant characteristics were investigated. Assays of mushroom and cellular tyrosinase activities and melanin content of mouse melanoma cells were performed spectrophotometrically, and the expression of melanogenesis-related proteins was determined by Western blotting. The possible signaling pathways involved in essential oil-mediated depigmentation were also investigated using specific protein kinase inhibitors. *E. camaldulensis* flower essential oil inhibited melanogenesis through its antioxidant properties and by down-regulating both mitogen-activated protein kinases (MAPK) and protein kinase A (PKA) signaling pathways. The present study indicates that the essential oil has the potential to be developed into a skin care product [150].The free radical scavenging activities of the *Eucalyptus microtheca* essential oil were assessed by measuring their scavenging abilities for stable 2,21-diphenyl-1-picrylhydazyl DPPH radicals . The DPPH scavenging activity was high in E. microtheca (81.8%) [145, 151].

Eupatorium cannabinum

The DPPH antioxidant assay was used to determine the antioxidant effect of hydro-alcoholic extract of *Eupatorium cannabinum* (at doses of 0.1-10mg/ml). EC50 of the extract was determined at 2.91mg/ml [152].

The antioxidant activities of caffeoyl derivatives identified in the aerial parts of *Eupatorium cannabinum* subsp (ndividually): chlorogenic acid (EC_{50} = (13.80 ±0.36) μmol/l), 3,5-dicaffeoylquinic acid (EC_{50} = (7.62 ± 0.22) μmol/l), 1,5-dicaffeoylquinic acid (EC_{50} = (7.85 ±0.23 μmol/l) and 4,5-dicaffeoylquinic acid (EC_{50} = (7.99 ±0.31 μmol/l) [153-154].

Euphorbia hirta

The antioxidant activities of different parts (leaves, stems, flowers and roots) of *Euphorbia hirta* were studied by diphenyl-1-picrylhydrazyl (DPPH) assay and reducing power by cyanoferrate method. The leaves extract exhibited a maximum DPPH scavenging activity of (72.96±0.78)% followed by the flowers, roots and stems whose scavenging activities were (52.45±0.66)%, (48.59±0.97)%, and (44.42±0.94) %, respectively. The standard The IC_{50} for leaves, flowers, roots, stems and BHT were 0.803, 0.972, 0.989, 1.358 and 0.794 mg/ml, respectively. The reducing power of the leaves extract was comparable with that of ascorbic acid and found to

be dose dependent [155-156]. The leaves extract of *Euphorbia hirta* was investigated for antioxidant activity. The methanolic extract of *Euphorbia hirta* showed DPPH scavenging activity of 89.75±0.032% and hydroxyl radical scavenging activity of 83.5±0.046% at 100µg/ml, while ethyl acetate fraction sowed DPPH scavenging activity of 91.88±0.060% and hydroxyl radical scavenging activity of 85.53±0.023%. Ethyl acetate fraction was further used for in-vivo antioxidant activity. Ethyl acetate fraction showed significant in-vivo antioxidant activity, 1.207±0.10, 45.85 ±5.2, 0706±0.03 and 0.0106±0.005 for glutathione, superoxide dismutase, catalase activity and lipid peroxidation respectively [157].

The *E. hirta* methanol extract showed antioxidant activities (IC_{50} = 10.57 µg/ml , 2,2-diphenyl-1-picrylhydrazyl; 850.23 µg/ml, superoxide-anion radical scavenging activity and 23.63 mg gallic acid equivalent per gram extract) [158].

Euphorbia macroclada

Ethanolic extract from leaves of *E. macroclada* showed higher content in total phenolic and total flavonoid than aqueous extract and showed higher content in total phenolic and total flavonoid than found in the stems. Ethanolic extract showed higher antioxidant capacity than aqueous extract by using DPPH and H_2O_2 tests, but with the using the chelating of ferrous ions test, the antioxidant activity of the aqueous extract of both stems and leaves was stronger than that of ethanolic once [159].

The leaves and stems of this plant have exerted high antioxidant power at different concentrations. The DPPH test demonstrated that 2.4mg/ml of both stems and leaves of *E. macroclada* have significantly increased the % of scavenger activity by 82% and 94% respectively. the IC50 of DPPH was 1.37mg for the stems and 1.22mg for the leaves. At the same time, the IC50 of H2O2 was 0.73mg for the stems and 0.61mg for the leaves of *E. macroclada* [160].

The antioxidant activity of *Euphorbia macroclada* extracts was determined by β-carotene bleaching method, free radical scavenging activity by 1,1-diphenyl-2- picryl-hydrazil (DPPH) assay, and ferrous metal chelating activity based on ferrozine-Fe^{+2} complex. Petroleum ether extract showed IC_{50} (µg/ml) in DPPH system > 100, 71.83±0.93% Inhibition in β-carotene/linoleic acid system (50 µg/ml) and 21.26±0.79% Inhibition Ferrozine–Fe^{2+}system (50 µg/ml). Acetone extract showed IC_{50} (µg/ml) in DPPH system 55.24±1.29, 73.26±1.32 % Inhibition in β-carotene/linoleic acid system (50 µg/ml) and 7.82±0.14% Inhibition Ferrozine–Fe^{2+}system (50 µg/ml). Ethanol extract showed IC_{50} (µg/ml) in DPPH system 51.13±1.00, 86.13±0.18% Inhibition in β-carotene/linoleic acid system (50 µg/ml) and 3.26±0.26% Inhibition Ferrozine–Fe^{2+}system (50 µg/ml) [161].

II. CONCLUSION:

This review was designed to highlight the antioxidant effects and free radical scavenging activity of medicinal plants as a third part of our previous reviews.

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