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Evalution of Antioxidant Activity of Various Herbal Folk Medicines

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Abstract

The leaves of some plants are flamed and used to dress injured skin, to stimulate healing and to ward off infection. This study aims to determine antioxidant activities of *Plantago lanceolata* L., *Plantago major* L. ssp. *major, Rubus hirtus* Waldst& Kit., *Sambucus ebulus* L., *Morus alba* L. and *Hedera helix* L., which grown in Düzce and its surroundings and are used for anti-inflammatory purposes, and especially for wound-healing. The total phenolic compound of these plants was also evaluated. In order to determine the antioxidant compounds and antioxidant capacity of these plants, different in vitro methods. Resulting from our study, while *P. lanceolata, R. hirtus, S. ebulus* and *P.major* ssp. major had similarities in terms of phenolic compounds content, *M. alba* and *H. helix* were found to have relatively low content of phenolic compounds. In general, *Plantago* species and *R. hirtus* showed high antioxidant activities.

Keywords: Antioxidant activity; *Plantago major* ssp. *major; Plantago lanceolata; Rubus hirtus; Hedera helix; Morus alba; Sambucus ebulus*

Introduction

It has been established that oxidative stress is among the major causative factors in induction of ageing and immunosupression, and many chronic and degenerative diseases including atherosclerosis, ischemic heart diseases, diabetes mellitus, cancer, neurodegenerative diseases and others [1]. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retarding the lipid oxidative rancidity in foods [2]. Recent studies have investigated the potential of plant products as antioxidants against various diseases induced by free radicals [3].

There is an increasing interest in natural antioxidants, e.g, polyphenols, present in medicinal and dietary plants, which might help preventing oxidative damages [4]. It has been determined that the antioxidant effect of plant products is mainly attributed to phenolic compounds such as flavonoids and phenolic acids [5].

Plantago lanceolata and *Plantago major* are species of *Plantago* genus including 275 species throughout the world. Their leaves are used as astringent, anti-inflammatory, expectorant, topical anodyne, antibacterial, styptic, hemoptysis, hematuria, sore throats, coughs, diarrhea, dysentery, hemorrhoids, cervicitis, rectal fissures, insect bites, snake bites, cuts, bruises and abscess. These *Plantago sp*. consist several compounds, including iridoid glycosides (aucubin, catapol, gardoside, geniposidic acid, mayoroside, melittoside), terpenoids (loliolid, ursolic acid, oleanolic acid), caffeic acid derivatives (caffeic acid, chlorogenic acid, plantamajoside R, aceteoside R), polysaccharides (plantaglucide, glucomannon, PMII, PMIa), alkoloids (indicain, plantagonin), polyholozide, flavonoids and flavone glucosides (luteolin-7 glucoside, hispidulin 7-glucuronide, apigenin, balcalein, scutallarin, plantagonin) [6].

Rubus hirtus is commonly called as blackberry belonging to family Rosaceae. It is present and widespread in the Turkey. The leaves of *R.hirtus* are used for abscess and to stop bleeding in cuts [7] and contains quercetin, kaempferol, caffeic acid and chlorogenic acid), phenolic acids, tannins, amino acids, sugars, pectins, carboxylic acids, anthocyanins, catechins, vitamin C and saturated or unsaturated fatty acids [8-11].

Sambucus ebulus belong to family Caprifoliaceae. *S. ebulus* grows mainly in the north of Turkey. Its leaves used for the treatment of inflammatory reactions, such as hemorrhoid, bites and sore-throat. The phytochemical compounds of *S. ebulus* are flavonoids, steroids, tannins, glycosides, cardiac glycosides, caffeic acid derivatives, ebulitins, ebulin 1 and volatile substances [12-18]. Traces of a cyanogenic glucoside, sambunigrin and the triterpenes alpha- and beta-amyrin were isolated from leaves, roots and fruits.

Morus alba is known as white mulberry belonging to Moraceae family. Its leaves are used as a blood pressure depressant. Chemical constituents of *Morus alba* leaves are prenylflavanes, glycoside, isoquercitrin, astragalin, scopolin, skimmin, roseoside II and benzyl D-glucopyranoside [19].

Hedera helix, ivy, is a species of Hedera genus in the family Araliaceae, native to most of Europe and western Asia. *Hedera helix* leaves are used for wound healing, gout, rheumatism and externally against parasites. and to treat burns. The leaves of *Hedera helix* consist many aktive compounds such as hederagenin, oleanolic acid and bayogenin, α-hederin, hederagenin-3-O-ß-D-glucoside, hederasaponin C (hederacoside C) with other hederasaponins (B, D, E, F, G, H and I), flavonoids such as quercetin, kaempferol, isoquercitrin and astragalin, phytosterols as stigmasterol, sitosterol, cholesterol, campesterol, α-spinasterol, coumarin glycoside scopolin and the polyacetylenes falcarinone, falcarinol and 11, 12-dihydrofalcarinol [20].

Since ancient times, herbs have been routinely used to treat wounds, and in many cultures their use in traditional medicine has persisted to the present [21]. In Düzce, plants are often used for wound healing, stomach soothing, curing intestinal disorders and coughs, and for healing burns. The ways of using plants are generally the same: fresh

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leaves of plants, infusion, decoction, poultice and expressed juice from fresh plants.

The plants in this study were selected from among those used especially for wound-healing in Düzce and its surroundings. The study was designed to examine antioxidant characteristics of *Plantago lanceolata* L., *Plantago major* L. ssp. *major, Rubus hirtus* Waldst & Kit., *Sambucus ebulus* L., *Morus alba* L. and *Hedera helix* L. which used as anti-inflammatory. In addition, through assessing total phenolic contents of the plants aimed to investigate whether there is a relationship between phenolic content and antioxidant activity.

Materials and Methods

Chemicals

1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Aldrich), N, N-dimethyl-p-phenylenediamine dihydrochloride (DMPD) (Fluka), ferric chloride (Aldrich), amonium acetate (Fluka), 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid diammonium salt) (ABTS) (Sigma), linoleic acid (Fluka), Tween 20 (Sigma), ethanol (Merck), methanol (Merck), ammonium thiocyanate (Sigma & Aldrich), nicotinamide adenine dinucleotide (NADH) (Sigma), nitro blue tetrazolium chloride (NBT) (Sigma), phenazine methosulphate (PMS) (Sigma), 30% hydrogen peroxide (Fluka), Folin-Ciocalteu reagent (Sigma & Aldrich), sodium carbonate (Na_2CO_3) (Sigma &Aldrich), sodium hydroxide (NaOH) (Merck), copper(II)sulfate ($CuSO_4$) (Sigma & Aldrich), sodium potassium tartrate ($NaKC_4H_4O_6$) (Sigma & Aldrich), Total Antioxidant Response kit (Abbott).

Plant material and extraction

P. lanceolata, P. major ssp. *major, R. hirtus, S. ebulus, M. alba and H. helix* were collected from Düzce and its surroundings. Plants were dried in the shadow for extraction. Plants were collected in June. The purposes of use of plants in this region are given at the Table 1.

Plant leaves were extracted with 50% (w/v) ethanol and methanol and bidistilled water. A mass of 1 g of dry herb was added to 15 ml of solvent and was stirred up in a glass flask tightly closed for 45 minutes. Then, 5 ml of solvent was added and stirred up for another 45 min;. Finally 5 ml of solvent was added and stirred up for a final 15 min (totalling 105 min). First, the herb extracts were filtered out using a fine textured cloth. Then, the extracts were filtered out of a filter paper. All studied plant extracts were portioned and stored at -80°C [22].

Folin-Ciocalteu method for total phenolic content

The amount of total soluble phenolics was determined according to the Folin-Ciocalteu method [23], modified for use by a plate reader. Folin Ciocalteu's phenol reagent was diluted to a volume ratio of 1:3 with 96% deionised water before used. Lowry A solution was prepared from sodium carbonate so that the strength (w/v) of Na_2CO_3 in 0.1 M NaOH solution was 2%. Lowry B solution was prepared from copper(II) sulphate so that the strength (w/v) of $CuSO_4$ in 1% sodium potassium tartrate (NaKC4H4O6) solution was 0.5%. Lowry C solution was prepared by freshly mixing 50 ml Lowry A with 1 ml Lowry B. To 100 µl of herb extract, 125 µl Lowry C was added. The mixture was mixed on a microplate shaker at 250 rpm. Then, 12.5 µl Folin reagent was added and 30 min was allowed for stabilization and to turn blue in colour. The absorbance against a reagent blank was measured at 750 nm.

CUPRAC assay

Cuprac assay is based on reduction of Cu(II) to Cu(I) by antioxidants (reductant) which are present in the sample [24]. We modified this assay to work with 96-well plates. $CuCl_2$ solution, 1.0×10^{-2} M, was prepared by dissolving 0.17 g $CuCl_2.2H_2O$ in water, and diluting to 100 mL. Ammonium acetate buffer at pH 7.0, 1.0 M, was prepared by dissolving 19.27 g NH_4Ac in water and diluting to 250 mL. Neocuproine (Nc) solution, 7.5×10^{-3} M, was prepared daily by dissolving 0.039 g Nc in 96% ethanolic. 50 µl $CuCl_2$ solution (10^{-2} M), 50 µl neocuproine alcoholic solution (7.5×10^{-3} M) and 50 µl NH_4Ac buffer solution, 27.5 µl sample and 27.5 µl water were added to wells. The mixture is then mixed well and incubated the microwell strips at room temperature (18° to 25°C) for about 30 min in dark. After that, absorbance was measured against a reagent blank at 450 nm.

DPPH assay

The free radical scavenging activity of these plants were measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH.) method, modified for working with 96 well plate. With this method it was possible to determine the antiradical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. As a result of the color changing from purple to yellow the absorbance is decreased when the DPPH radical is scavenged by an antioxidant through donation of hydrogen to form a stable DPPH-H molecule [25]. This method was applied to ethanolic, methanolic and aqueous extracts of plants (1000-500-250-125 µg/ml). First, 0.1 mM solution of DPPH· in MeOH was prepared. Then, 50 µl of this solution was added to 150 µl of extracts of plants. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in an ELISA reader. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity [26]. The DPPH· scavenging effect calculated using following equation:

= [Abscontrol-Abssample/Abscontrol]×100

Plant name	Part used	Use
Plantago lanceolata L. (Plantaginaceae)	Leaf	For stomach ache, intestinal disorders, , internal disorders, maturation of an abscess, shortness of breath, diabetes, wound healing and to treat burns.
Plantago major L. subsp. *major* (Plantaginaceae)	Leaf	For the maturation of an abscess, hemorrhoids , kidney cancer, wound healing, urinary infection, bronchitis, cough ,diarrhea, stomach bleeding, ulcer, stomach ache and as a panacea.
Rubus hirtus Waldst & Kit. (Rosaceae)	Leaf	For cuts, healing wound, anemia and to treat stomach ache.
Sambucus ebulus L. (Caprifoliaceae)	Leaf	For bee stings, pain, vaginal pain after birth, wound healing, malaria and to treat swellings of breasts in cattle, rheumatic pain.
Morus alba L. (Moraceae)	Leaf	For wound healing.
Hedera helix L. (Araliaceae)	Leaf	For wound healing and to treat burns.

Table 1: Folk Remedies in Düzce.

DMPD assay

DMPD assay modified for use by a plate reader. DMPD (100 mM) was prepared by dissolving 209 mg of DMPD in 10 ml of deionised water. This solution (1 ml) was added to 100 ml of 0.1 M acetate buffer, pH 5.25, and the coloured radical cation (DMPD+) was obtained by adding 0.2 ml of a 0.05 M solution of ferric chloride (the final concentration was 0.01 mM). This solution (225 µl) was transferred directly to the microwell and its absorbance at 505 nm was measured (A0). Samples (15 µl) were added to all wells. Then added 210 µl of DMPD+ to all samples and, stirred and left to stand for 10 min. After this period, a decrease in absorbance was measured (A1). The DMPD. solution was used as control [27].

The $DMPD^+$ scavenging effect calculated using following equation:

$$= [A_0 - A_1/A_0] \times 100$$

ABTS assay

ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS.) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark, at room temperature for 12-16 h before use. The kept solution was diluted with 96% ethanolic at a ratio of 1: 30. Respectively 10 µl sample, 200 µl ABTS. was added to wells. 210 µl of ABTS. solution was used as control (A0). Then, the change in absorbance during 5 min was recorded and measured at 734 nm by an ELISA reader [28].

The $ABTS^{\cdot}$ scavenging effect calculated using following equation:

$$= [A_0 - A_1/A_0] \times 100$$

Ferric thiocyanate assay

The reaction mixture consisted of 0.28 g linoleic acid, 0.28 g of Tween 20 and 50 ml of phosphate buffer (0.05 M, pH 7.4). To 1.25 ml of the above linoleic acid emulsion, 0.1 ml of test sample and 1.25 ml of phosphate buffer (0.2 M, pH 7.0) were added and incubated at 40°C for 24 h. The mixture prepared, as above, without the test sample, was the control. After 24 h, 0.1 ml of the mixture was taken and mixed with 4.7 ml of 75% ethanolic, 0.1 ml of 30% ammonium thiocyanate and 0.1 ml of 20 mM ferrous chloride in 3.5% HCl and allowed to stand at room temperature. Precisely 3 min after the addition of ferrous chloride to the reaction mixture, the absorbance at 500 nm was measured [29].

Scavenging of superoxide

The reaction mixture, which contained 50 µL of sample, 50 µL of NADH (936 µM) in phosphate buffer (100mM, pH 7.4), 50 µL of NBT (300 µM) in phosphate buffer and 50 µL of PMS in phosphate buffer was incubated at room temperature for 5 min. The absorbance was read at 560 nm by an ELISA reader, against blank samples (A_1). The reaction mixture, without the extract was used as control (A_0) [30].

The superoxide scavenging effect calculated using following equation:

$$= [A_0 - A_1/A_0] \times 100$$

Scavenging of hydrogen peroxide

A solution (43 mM) of hydrogen peroxide was prepared in phosphate buffer (0.1 M pH 7.4). 400 µL of sample was mixed with 960 µL phosphate buffer. Then, 240 µL of hydrogen peroxide solution (43 mM) was added to the mixture. Absorbance was read at 230 nm by a spectrophotometer against blank samples (A_1). The reaction mixture, without the extracts was used as control (A_0) [31].

The hydrogen peroxide scavenging effect calculated using following equation:

$$= [A_0 - A_1/A_0] \times 100$$

Total Antioxidant Response (TAR)

To 5 µl herbal extract, 200 µl R1 (o-dianisidine) was added. The first absorbance was taken before mixing R1 and R2 (as sample blank). After 4,5 min, 10 µl of R2 (H_2O_2) was added the mixture and the last absorbance was taken when the reaction trace drew a plateau line (about 3-4 min after the mixing). Measurements were taken by an Elisa reader at 444 nm [32].

Results

On the basis of these mechanisms, the present study was designed to examine the antioxidant capacity by eight commonly used methods of some plants, which are considered to have anti-inflammatory effects. Determination of total phenolic content, DPPH and DMPD radical scavenging, cupric ion (Cu(2+)) reducing, inhibition lipid peroxidation, hydroxyl radical scavenging capacity were applied to the plants which are collected from Düzce and its surroundings and used as folk medicine. As far as we know, Folin, Cuprac, and DPPH methods were modified for the first time for our study, to work with 96 well plates.

Folin-Ciocalteu method for total phenolic content

The total phenolic contents of the plants were determined from the regression equation of gallic acid calibration curve (y=0.4685 ln(x)–1.131) (Figure 1). The concentrations of total phenolic content of extracts of plants are shown in Table 2. The highest amount was found in the *P. lanceolata*, followed by *R. hirtus, S. ebulus, P. major* ssp. *major, H. helix* and *M. alba*, respectively.

CUPRAC assay

Cuprac is one of the assays to determine the total antioxidant activity, which uses cupper II as an oxidant. This method is based on the reduction of Cu(II) to Cu(I) by antioxidants (reductant), which present in the sample. This method measures the capacity of hydrophilic and lipophilic antioxidants. On the first trial, standards gave good results and the same results were obtained from extracts. Trolox (a water-soluble tocopherol analogue), gallic acid and vitamin C were used as standard solutions. We observed a good correlation between the concentration and percentage inhibition(y=0.0032 ln (x)-0.0379, R^2=0.9985 for trolox, y=0.4685 ln (x)-1.131, R^2=0.9893 for gallic acid, y=0.0024 ln (x)-0.2221, R^2=0.9695 for vitamin C). According to the results of the cupper reducing antioxidant capacity (CUPRAC) assay, *R. hirtus* and *P. lanceolata* showed the highest antioxidant activity, *M. alba* showed the lowest activity in aqueous and methanolic extracts (Figure 1a and 1b). In ethanolic extracts, while *P. lanceolata* exhibited the

Aqueous extracts (1 mg/mL)	Total phenolic content (µg/mL)
Plantago lanceolata L.	146.368
Rubus hirtus Waldst&Kit	144.814
Sambucus ebulus L.	135.542
Plantago major L. ssp. *major*	107.407
Hedera helix L.	39.555
Morus alba L.	13.721

Table 2: Total phenolic content of plants.

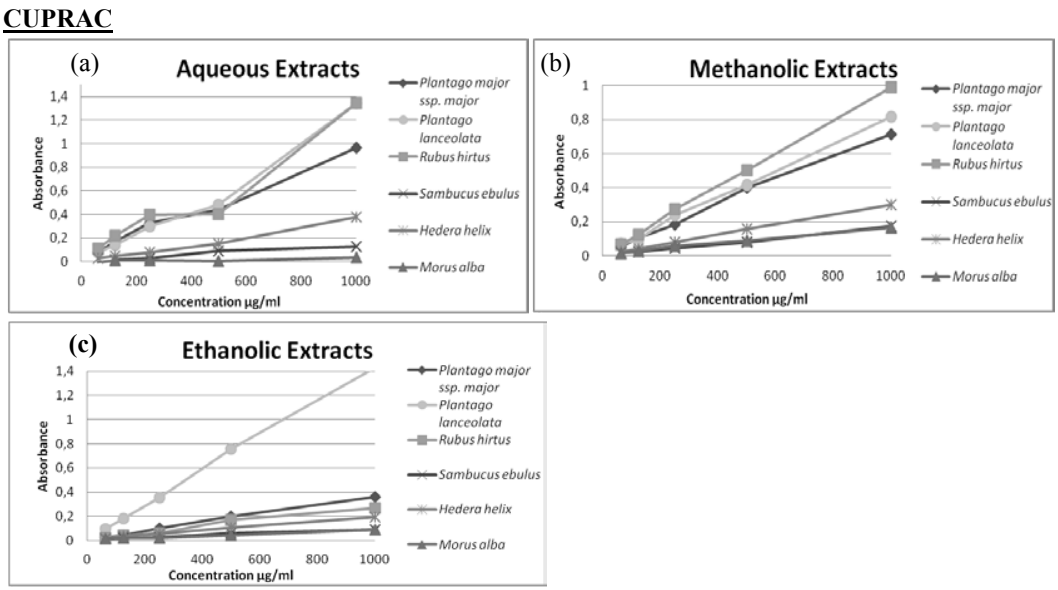

Figure 1: Antioxidant activities of plant extracts determined using cuprac method.

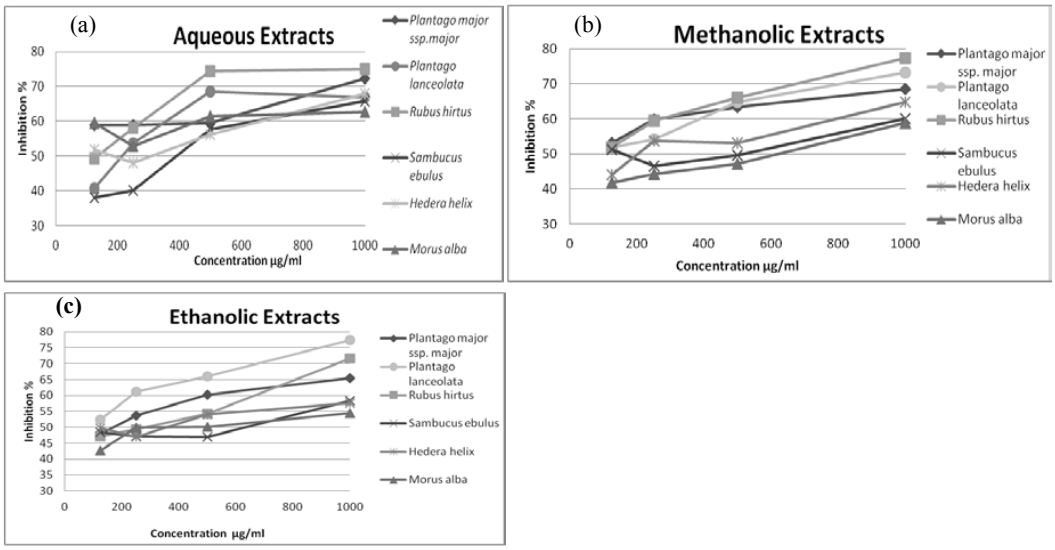

Figure 2: Scavenging of DPPH radical solution by extracts having different concentrations and solvents.

highest antioxidant activity, *M. alba* again showed the lowest activity (Figure 1c).

DPPH assay

DPPH free radical scavenging is one of the generally accepted mechanisms against lipid oxidation. Difference between DPPH free radical binding method and the other method is the short run time allowing rapid determination of the radical scavenging. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. The antioxidant activity of plant extracts were calculated according to the percentage inhibition in DPPH assay. Gallic acid was used as the reference standard (y=0.9221 ln (x)+01.039, R^2=0.9925). The DPPH free radical scavenging activity of the plant extracts are shown in Figure 2a-2c. While *R. hirtus* had the highest activity in aqueous and methanolic extracts, *P. lanceolata* showed the highest DPPH free radical scavenging activity in ethanolic extracts. *M. alba* showed the lowest DPPH free radical scavenging activity in aqueous, methanolic and ethanolic extracts.

DMPD assay

DMPD method is suitable for fast and sensitive measurement of antioxidant activity of hydrophilic compounds. In our study, although we observed high correlations in DMPD radical and Trolox standard studies, we couldn't reach the same consistency in all plant extracts. However, similar results to those in other methods were observed in methanolic extracts gave the most uniform results. Different

concentration of DMPD was carried out (y=0.1339 ln (x)-0,083, R^2=0.9875) and trolox was used as standard (y=17.771 ln (x)-63.376, R^2=0.9901). The antioxidant activity of plant extracts were calculated according to the percentage inhibition in DMPD assay. According to our results *R. hirtus* gave the highest antioxidant activity in methanolic extracts, *P. major* ssp. *major* gave the highest antioxidant activity in aqueous extracts and *P. lanceolata* gave the highest antioxidant activity in ethanolic extracts (Figure 3a and 3c). While *S. ebulus* had the lowest antioxidant capacity in ethanolic extracts, *M. alba* showed the lowest antioxidant capacity in methanolic and aqueous extracts (Figure 3b).

ABTS assay

ABTS method is commonly used to assess radical scavenging or antioxidant activity of the sample on the inhibition of ABTS radical (ABTS·), which is a synthetic compound and is produced by oxidation of ABTS. ABTS assay is based on the decolourization of blue- green ABTS radial cation, which is reduced by antioxidant compounds, plant, and fruit extracts. Trolox, which was observed to have a good correlation between the concentration and percentage inhibition (y=-0.0001 x^2+0.2374x-3.2115, R^2=0.9953), was used as standard. According to our results, *P. lanceolata*, *P. major* ssp. *major*, *R. hirtus*, and *S. ebulus* gave almost the same antioxidant activity, *M. alba* showed the lowest activity in aqueous extracts (Figure 4a). Outside the small differences, we observed roughly similar results in methanolic and ethanolic extracts (Figure 4b and 4c).

Ferric thiocyanate assay

In our study, inhibition of lipid peroxidation capacity of aqueous

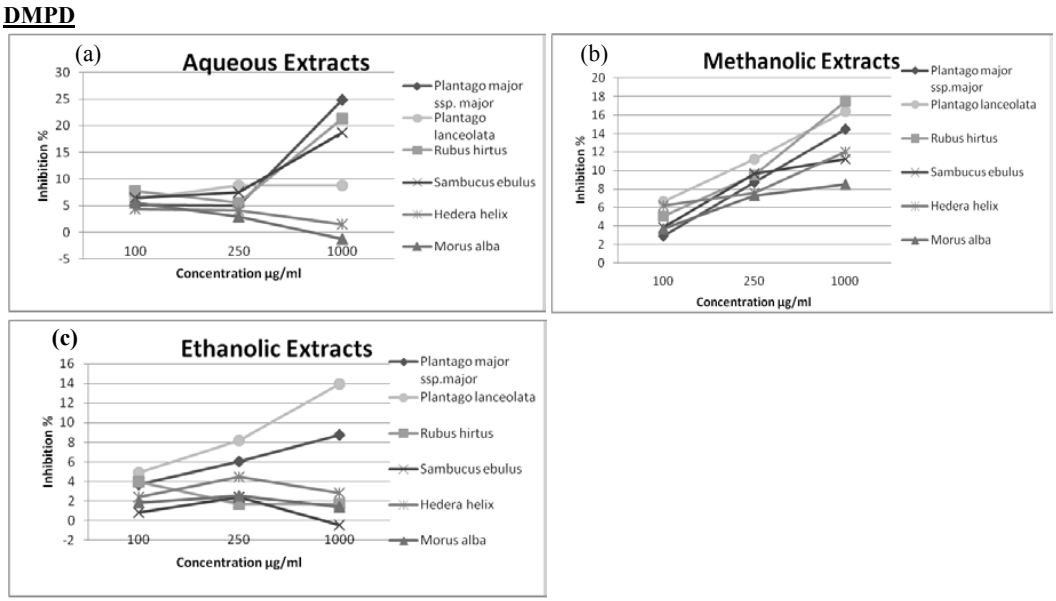

Figure 3: Scavenging of DMPD radical solution by extracts having different concentrations and solvents.

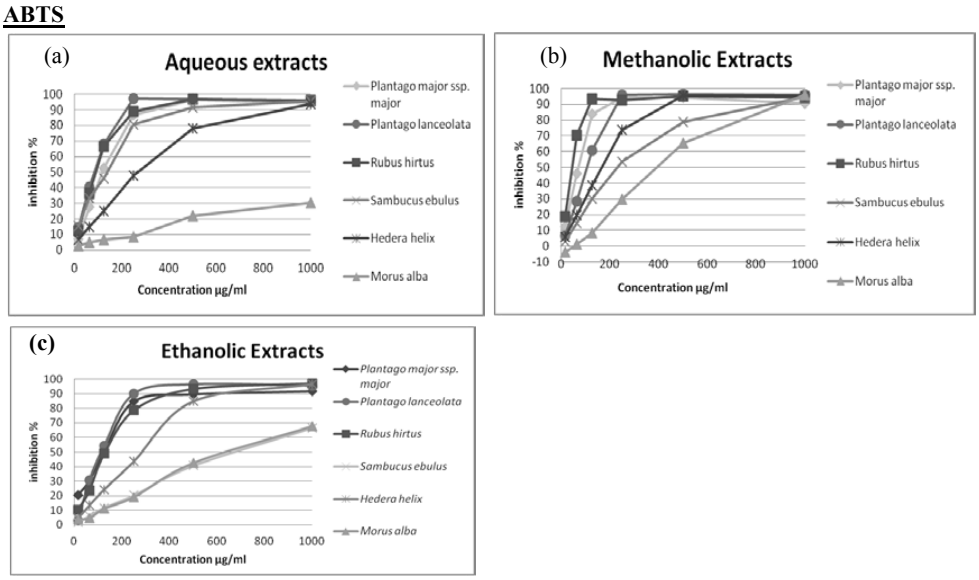

Figure 4: Scavenging of ABTS radical solution by extracts having different concentrations and solvents.

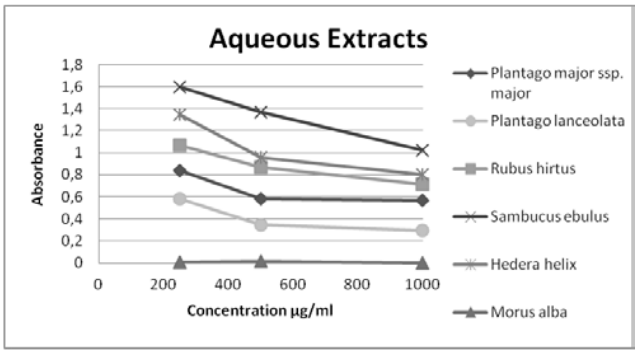

Figure 5: Total antioxidant activities of aqueous extracts of plants was determined by ferric thiocyanate method in linoleic acid emulsion.

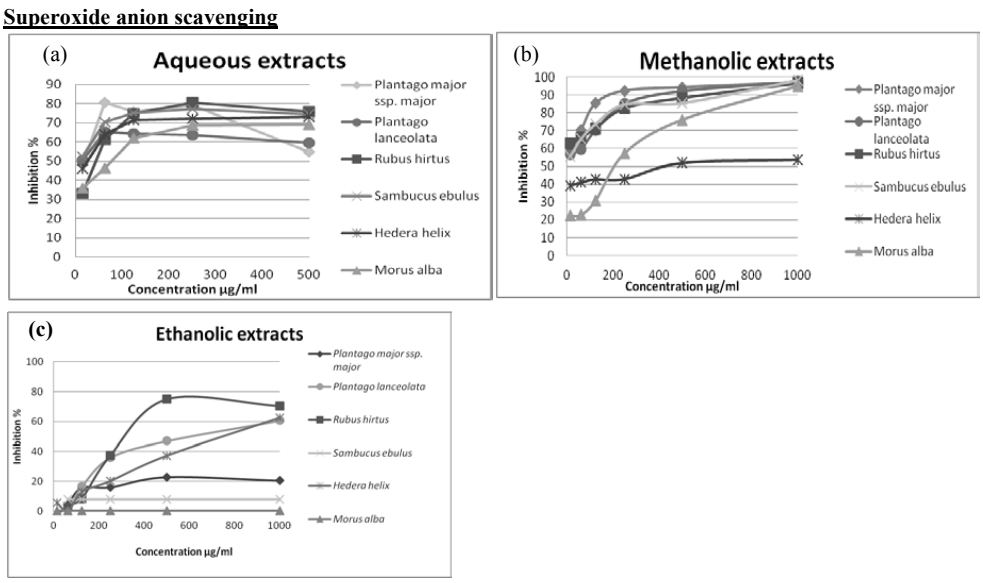

Figure 6: Superoxide anion scavenging activity by extracts having different concentrations and solvents.

plants were investigated by ferric thiocyanate assay, which is based on the oxidation of fats and oxidizing Fe^{+2} to Fe^{+3} by peroxides and Fe^{+3} ions which occur in this reaction, making it rather complex with SCN. Decrease in the absorbance indicates an increase in the free radical scavenging activity. In this assay, gallic acid was used as standard (y=-0.224 ln (x)+1.6333, R^2=0.9673). The results showed that *P. lanceolata* had the highest antioxidant activity followed by *P.major* ssp. *major, R. hirtus, H. helix,* and *S. ebulus*. The present work demonstrated that the aqueous extract of *M. alba* leaves did not show remarkable activity (Figure 5).

Scavenging of superoxide

Superoxide (O_2^{-}•) is accepted to be a very harmful radical that can be converted into more reactive species, such as hydroxyl radical or peroxynitrite, contributing to tissue damages and various diseases. The superoxide radical assay of samples was measured according to the method reported by Robak and Gryglewski [30]. The scavenging activity of superoxide radical of plant extracts was measured and the percentage inhibition was calculated. Different concentration of vitamin C and gallic acid used as standard (y=-6E-05 x^2+0.109x+25.947 for vitamin C, R^2= 0.9994 and y=12.485 ln (x)+2.186, R^2=0.9848 for gallic acid). According to the data *R. hirtus* gave the highest antioxidant activity and *M. alba* showed the lowest activity in aqueous and ethanolic extracts (Figure 6a and 6c). In methanolic extracts, while *R. hirtus* exhibited the highest antioxidant activity, *H. helix* showed the lowest activity (Figure 6b).

Scavenging of hydrogen peroxide

Hydrogen peroxide (H_2O_2) is a weak oxidizing agent but it can cross cell membranes rapidly. Once inside the cell, H_2O_2 can react with Fe^{+2} ions to generate the hydroxyl radical and this may be the origin of many of its toxic effects [33]. H_2O_2 is relatively stable in the absence of reducing compounds. Scavenging of H_2O_2 by plant extracts may be attributed to their electron donating abilities [34]. Hydrogen peroxide scavenging activity of the plant extracts is presented in Figure 7; In this method, we used only one concentration (50 µg/mL) of all extracts. In aqueous extracts, *M. alba* showed the highest activity and *P. lanceolata* showed the lowest activity. While *S. ebulus* gave the highest antioxidant activity, *P. lanceolata* showed lowest activity in methanolic and ethanolic extracts.

Total antioxidant response (TAR)

In the last method, to measure total antioxidant response, the

Hydrogen Peroxide

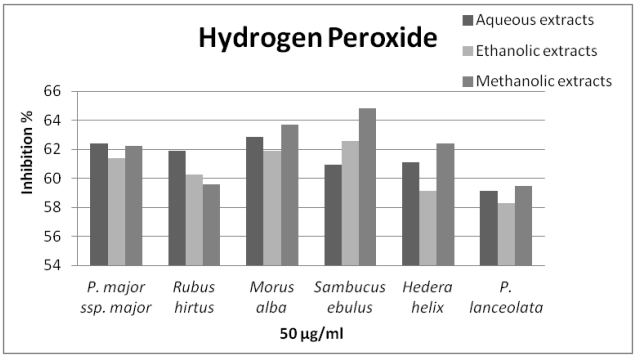

Figure 7: Hydrogen peroxide scavenging activites of 50 µg/ml concentration of plants.

TAR

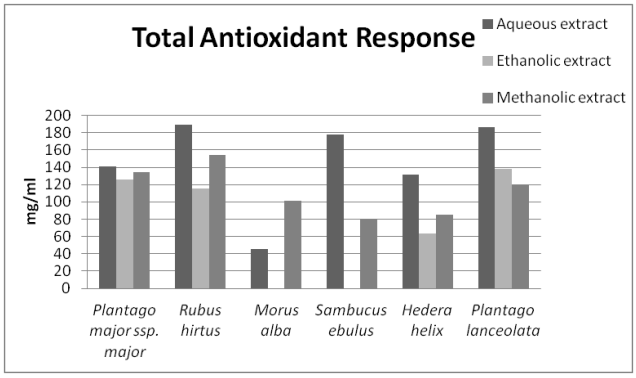

Figure 8: Total antioxidant capacity in plants (trolox-equivalent mg/ml).

hydroxyl radical is produced by the Fenton reaction and oxidize colourless substrate O-dianisidine to the dianisyl radical, which is bright yellowish-brown in colour. This method is based on suppression the colour formation by antioxidants, which are present in the sample. Results were expressed as trolox equivalent in mg of Trolox per ml ($y=-0.0028 \ln(x)+0.8247$, $R^2=0.9971$). When we examined the Trolox-equivalent antioxidant capacity of the samples, *R. hirtus* and *P. lanceolata* showed the highest activity, *M. alba* showed the lowest activity in aqueous extracts. While *Plantago* species showed the highest activity, *S. ebulus* and *M. alba* did not show any activity in ethanolic extracts. In methanolic extracts, *R. hirtus* showed the highest activity, *H. helix* and *S. ebulus* showed lowest activity. The ethanolic extracts of *S. ebulus* and M. alba did not give any result (Figure 8), this might be due to insufficient amount of the kit, therefore experiment cannot be replicate. The results, which are obtained by TAC method, are consistent with other experiments.

Discussion

The process of inflammation may be initiated by an infection or cell damage that stimulates the secretion of interleukins or tumour necrosis factor by macrophages. Activated neutrophils produce large amounts of ROS, thus causing the production of toxic reactive species to increase. Neutrophils also release myeloperoxidase (MPO). Myeloperoxidase (MPO) catalyzes the reaction of hydrogen peroxide with chloride ion to produce hypochlorous anion. Also reaction of hypochlorous anion with hydrogen peroxide yields singlet oxygen, which is a reactive species. The interaction of superoxide radical with hydrogen peroxide and iron is causing the formation of hydroxyl radical, which is highly reactive, and the reaction of superoxide with nitric oxide is resulting in the formation of peroxynitrite. These reactive species kill pathogens by halogenations or by protein oxidation and lipid peroxidation. If the balance between oxidants and antioxidants shifts in the direction of oxidants during the inflammation, healthy cells can get damaged [35,36].

Plant-derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins and proanthocyanins could delay or prevent the onset of degenerative diseases because of their redox properties, which allow them to act as hydrogen donors, reducing the agents, hydroxyl radicals (OH.) or superoxide radical ($O_2^{\cdot}$) scavengers [37,38].

Phenolic compounds are herbal substances whose chemical structures may range from quite simple compounds to highly polymerized substances. The capacity of phenolics to scavenge free radicals may be due to many phenolic hydroxyl groups they posses. Phenols play an important role in antioxidant activity, because they transfer hydrogen to radicals and produce phenoxide radical, which is stabilized. Therefore, it is important to determine the total amount of phenolics to determination of antioxidant capacity of plants. In our study, we found higher phenolic content in *P. lanceolata, R. hirtus, S. ebulus and P. major* ssp. *major* than those in *H. helix* and *M. alba*.

Plantago genus contains 275 species throughout the world and has been used for medical purposes since ancient times. In recent years, more research has been devoted to the study of antioxidants for these plants, which are astringent, anti-toxic, antimicrobial, anti-inflammatory, expectorant and diuretic. Some of the compounds in Plantago sp. such as ursolic acid, plantamajoside R, aceteoside R and plantagonin have been shown to have both an antioxidant and anti-inflammatory effect [6]. Numerous studies have shown that *Plantago* species had a high phenolic content and antioxidant activity [39-48]. There are few studies carried out in Turkey showing high antioxidant activity for *P. lanceolata* [40-50] and *P. major* [51,52]. In our study, we also observed high content of phenolic compounds and antioxidant activity for *P. major* ssp. *major* and *P. lanceolata* in some of the methods.

In several studies, very high antioxidant activities were observed in different types of *Rubus* (*Rubus urticifolius* Poir., *Rubus parviflorus* Nutt.) [53-57]. Although anti-inflammatory and antinociceptive effects of *R. hirtus* have shown in previous studies [58,59], only one study has specifically investigated the antioxidant activity of its fruit [60]. As far as we know, this is the first study showing antioxidant activity in the leaves of *R. hirtus*. According to our results, its antioxidant activities were mostly higher than other plants studied and we think that these high antioxidant activities of *R. hirtus* may contribute to its anti-inflammatory effects.

S. ebulus has shown high antioxidant activity in generally and a positive correlation was observed between antioxidant activity and phenolic content [61,62]. According to our results, although the phenolic content of *S. ebulus* was close to *P. lanceolata* and R. hirtus, their antioxidant properties were not similar. However, high levels of thiocyanate found in *S. ebulus* indicate its ability to inhibit lipid peroxidation. According to our knowledge, this is the first study, which has investigated the antioxidant activity of *S.ebulus* in Turkey.

It has been shown that alpha hederin and hedera saponin C component of *H. helix* had a strong total antioxidant activity and inhibits lipid peroxidation [63-67]. However, *H. helix* showed lower total phenolic content and lower antioxidant activity when compared

to other plants in our study. Similarly, in previous studies, leaves [68-70], fruit [71-73] and bark [74-76] extracts of *M. alba* showed high antioxidant activity. In our study, lowest phenolic compounds and antioxidant activity was observed in *M. alba*.

It is thought to be due to the different time and collection zone of study materials. In a previous study, Sivaci and Sökmen showed seasonal changes in antioxidant activity and total phenolic contents in stems of Morus species [77]. According to their results, *Morus* species had the highest antioxidant activity and total phenolic contents in October whereas the lowest in February, even in June, which is when we collected them. The similar seasonal changes in the contents of *H. helix* leaves have been shown by Fischer and Feller [78].

In conclusion, the aim of this study was to examine the antioxidant properties of some plant species chosen due to their anti-inflammatory uses and to obtain results can be useful for further studies. In our study, showing high antioxidant activity of the plant species with high phenolic content supports many other studies, which have shown an association between phenolic compounds and antioxidant activities. Because seasonal changes affect the contents of plants too, it is difficult to compare the activities of different plants. Observation of different antioxidant activities of some plant species which have been demonstrated their anti-inflammatory effect in the literature and used by people for this purpose can be interpreted in different ways. Because of their use for different purposes other than anti-inflammatory effects, and knowing their major effects makes it easy to comment on the results. For example, *S. ebulus* has been mostly used as a pain killer, as an anti-inflammatoir and has showed a relatively lower antioxidant activity in our study. In addition, if we had looked for their anti-inflammatory activities, it could be useful for the interpretation of such results.

Although it is not our main goal, we modified Folin, Cuprac, and DPPH methods to work with 96 well plates. This would be useful for those who will work with these methods.

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References

1. Young IS, Woodside JV (2001) Antioxidants in health and disease. J Clin Pathol 54: 176-186.
2. Pryor WA (1991) The antioxidant nutrients and disease prevention--what do we know and what do we need to find out? Am J Clin Nutr 53: 391S-393S.
3. Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, et al. (2003) Free radical-scavenging activity of Taiwanese native plants. Phytomedicine 10: 170-175.
4. Silva BA, Ferreres F, Malva JO, Dias ACP (2005) Phytochemical and antioxidant characterization of Hypericum perforatum alcoholic extracts. Food Chem 90: 157-167.
5. Pietta PG (2000) Flavonoids as antioxidants. J Nat Prod 63: 1035-1042.
6. Kuhn MA, Winston D (2000) Herbal therapy supplements ascientific traditional approach. Lippincott, New York.
7. Yeşilada E, Ustün O, Sezik E, Takaishi Y, Ono Y, et al. (1997) Inhibitory effects of Turkish folk remedies on inflammatory cytokines: interleukin-1alpha, interleukin-1beta and tumor necrosis factor alpha. J Ethnopharmacol 58: 59-73.
8. Tzouwara-Karayanni SM and Philianos SM (1981) Chemical constituents of Rubus ulmifolius Schott Q J Crude. Drug Res 19: 127-130
9. Rommel A, Wrolstad RE (1993) Ellagic acid content of red raspberry juice as influenced by cultivar, processing, and environmental factors. J Agric Food Chem 41: 1951-1960.
10. Gudej J, Tomczyk M (2004) Determination of flavonoids, tannins and ellagic acid in leaves from *Rubus* L. species. Arch Pharm Res 27: 1114-1119.
11. Tomczyk M, Gudej J (2005) Polyphenolic compounds from *Rubus saxatilis*. Chem Nat Com 41: 349-351.
12. Shokrzadeh M, Saeedi Saravi SS (2009) The chemistry, pharmacology and clinical properties of *Sambucus ebulus*: A review. J Med Plants Res 4: 95-103.
13. Ebrahimzadeh MA, Mahmoudi M, Karami M, Saeedi S, Ahmadi AH, et al. (2007) Separation of active and toxic portions in *Sambucus ebulus*. Pak J Biol Sci 10: 4171-4173.
14. Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M, Saremi S (1998) Antinociceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats. J Ethnopharmacol 61: 229-235.
15. Ghannadi AR, Ghassemi-Dehkordi N (1997) Pharmacognostical Investigations on *Sambucus ebulus* L. and *Sambucus nigra* L. DARU Journal of Pharmaceutical Sciences 7: 55.
16. de Benito FM, Citores L, Iglesias R, Ferreras JM, Soriano F, et al. (1995) Ebulitins: a new family of type 1 ribosome-inactivating proteins (rRNA N-glycosidases) from leaves of *Sambucus ebulus* L. that coexist with the type 2 ribosome-inactivating protein ebulin 1. FEBS Lett 360: 299-302.
17. Yesilada E (1995) Evaluation of the anti-inflammatory activity of the Turkish medicinal plant *Sambucus ebulus*. Chem Nat Comp 33: 539-540.
18. Pribela A, Durcanska J, Piry J, Karovicova J (1992) Volatile substances of dwarf elder *Sambucus ebulus* L. fruits Biologia. (Bratislava) 47: 225-230.
19. Doi K, Kojima T, Makino M, Kimura Y, Fujimoto Y (2001) Studies on the constituents of the leaves of *Morus alba* L. Chem Pharm Bull (Tokyo) 49: 151-153.
20. Lutsenko Y, Bylka W, Matlawska I, Darmohray R (2010) Hedera helix as a medicinal plant. Herba Polonica 56: 83-96.
21. Miller AG, Morris M, Stuart S (1988) Plants of Dhofar the southern region of Oman: traditional, economic and medicinal uses.
22. Yildiz L (2007) Spectrophotometric and chromatographic determination of antioxidant capacity in some plant samples [dissertation]. Istanbul University, Istanbul.
23. Apak R, Güçlü K, Ozyürek M, Esin Karademir S, Erçağ E (2006) The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. Int J Food Sci Nutr 57: 292-304.
24. Apak R, Güçlü K, Ozyürek M, Karademir SE (2004) Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem 52: 7970-7981.
25. Matthäus B (2002) Antioxidant activity of extracts obtained from residues of different oilseeds. J Agric Food Chem 50: 3444-3452.
26. Oktay M, Gulcin I, Kufrevioglu OI (2003) Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Food Science and Technology 36: 263-271.
27. Fogliano V, Verde V, Randazzo G, Ritieni A (1999) Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J Agric Food Chem 47: 1035-1040.
28. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26: 1231-1237.
29. Suja KP, Jayalekshmy A, Arumughan C (2005) Antioxidant activity of sesame cake extract. Food Chem 91: 231-219.
30. Robak J, Gryglewski RJ (1988) Flavonoids are scavengers of superoxide anions. Biochem Pharmacol 37: 837-841.
31. Ruch RJ, Cheng SJ, Klaunig JE (1989) Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10: 1003-1008.
32. Erel O (2004) A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 37: 112-119.
33. Halliwell B, Gutteridge JMC (1991) Free Radicals in Biology and Medicine. Second Edition. Clarendon Press, Oxford.
34. Wettasinghe M, Shahidi F (2000) Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. Food Chem 70: 17-26.

35. Ogino T, Packer L, Maguire JJ (1997) Neutrophil antioxidant capacity during the respiratory burst: loss of glutathione induced by chloramines. Free Radic Biol Med 23: 445-452.

36. Kukreja RC, Janin Y (1997) Reperfusion Injury: Basic Concepts and Protection Strategies. J Thromb Thrombolysis 4: 7-24.

37. Govindarajan R, Vijayakumar M, Pushpangadan P (2005) Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. J Ethnopharmacol 99: 165-178.

38. Robards K, Prenzler PD, Tucker G, Swaitsitang P, Glover W (1999) Phenolic compounds and their role in oxidative processes. Food Chem 66: 401-436.

39. Kardosová A, Machová E (2006) Antioxidant activity of medicinal plant polysaccharides. Fitoterapia 77: 367-373.

40. Herold A, Cremer L, CalugÄƒru A, Tamaay V, Ionescu F, et al. (2003) Antioxidant properties of some hydroalcoholic plant extracts with antiinflammatory activity. Roum Arch Microbiol Immunol 62: 217-227.

41. Vigo E, Cepeda A, Gualillo O, Perez-Fernandez R (2005) In-vitro anti-inflammatory activity of Pinus sylvestris and Plantago lanceolata extracts: effect on inducible NOS, COX-1, COX-2 and their products in J774A.1 murine macrophages. J Pharm Pharmacol 57: 383-391.

42. Gálvez M, Martín-Cordero C, Houghton PJ, Ayuso MJ (2005) Antioxidant activity of methanol extracts obtained from Plantago species. J Agric Food Chem 53: 1927-1933.

43. Samuelsen AB (2000) The traditional uses, chemical constituents and biological activities of *Plantago major* L. A review. J Ethnopharmacol 71: 1-21.

44. Korogodina VL, Bamblevskiĭ VP, Grishina IV, Gustova MV, Zabaluev SA, et al. (2000) The antioxidant status of the seeds in populations of the great plantain *(Plantago major* L.) growing in an area exposed to the Balakovo Atomic Electric Power Station and to chemical enterprises. Radiats Biol Radioecol 40: 334-338.

45. Chiang LC, Chiang W, Chang MY, Ng LT, Lin CC (2002) Antiviral activity of Plantago major extracts and related compounds *in vitro*. Antiviral Res 55: 53-62.

46. Park BG, Lee HS, Jung SH, Hong CO, Won HJ, et al. (2007) A 90 day repeated oral toxicity study on plantamajoside concentrate from Plantago asiatica. Phytother Res 21: 1118-1123.

47. Campos AM, Lissi EA (1995) Evaluation of the antioxidant capacity of herbal teas by a procedure based on the bleaching of ABTS radical cations. Bol Soc Chil Quim 40: 375-381.

48. Beara IN, Lesjak MM, Jovin ED, Balog KJ, Anackov GT, et al. (2009) Plantain (*Plantago* L.) species as novel sources of flavonoid antioxidants. J Agric Food Chem 57: 9268-9273.

49. Dalar A, Konczak I (2013) Phenolic contents, antioxidant capacities and inhibitory activities against key metabolic syndrome relevant enzymes of herbal teas from Eastern Anatolia. Ind Crop Prod 44: 383-390

50. Dalar A, Turker M, Konczak I (2012) Antioxidant capacity and phenolic constituents of Malva neglecta Wallr. and *Plantago lanceolata* L. from Eastern Anatolia Region of Turkey. J Herb Med 2: 42-51.

51. Kolak U, Boga M, Urusak EA, Ulubelen A (2011) Constituents of Plantago major subsp. intermedia with antioxidant and anticholinesterase capacities. Turk J Chem 35: 637-645.

52. Coban T, Citoglu GS, Sever B, Iscan M (2003) Antioxidant activities of plants used in traditional medicine in Turkey. Pharm Biol 41: 608-613.

53. Gao J, Sun CR, Yang JH, Shi JM, Du YG, et al. (2011) Evaluation of the hepatoprotective and antioxidant activities of *Rubus parvifolius* L. J Zhejiang Univ Sci B 12: 135-142.

54. Argoti JC, Salido S, Linares-Palomino PJ, Ramírez B, Insuasty B, et al. (2011) Antioxidant activity and free radical-scavenging capacity of a selection of wild-growing Colombian plants. J Sci Food Agric 91: 2399-2406.

55. Serteser A, Kargioğlu M, Gök V, Bağci Y, Ozcan MM, et al. (2008) Determination of antioxidant effects of some plant species wild growing in Turkey. Int J Food Sci Nutr 59: 643-651.

56. Ferreira DS, De Rosso VV, Mercadante AZ (2010) Compostos Bioativos Presentes Em Amora-Preta (*Rubus Spp.*). Rev Bras Frutic Jaboticabal 32: 664-674.

57. Schmeda-Hirschmann G, Feresin G, Tapia A, Hilgert N, Theoduloz C (2005) Proximate composition and free radical scavenging activity of edible fruits from the Argentinian Yungas. J Sci Food Agric 85: 1357-1364.

58. Akcos Y, Yesilada E, and Ezer N (1998) Anti-inflammatory activity of some Turkish Rubus species. Hacettepe University Journal of the Faculty of Pharmacy 18: 33-38.

59. Erdemoglu N, KÃ¼peli E, YeÅŸilada E (2003) Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine. J Ethnopharmacol 89: 123-129.

60. Guder A, Korkmaz H (2012) Investigation of antioxidant activity and total anthocyanins from blackberry (Rubus hirtus Waldst. and Kit) and cherry laurel (Laurocerasus officinalis Roem). Asian J Chem 24: 4525-4531.

61. Ebrahimzadeh MA, Nabavi SF, Nabavi SM (2009) Antioxidant activities of methanol extract of Sambucus ebulus L. flower. Pak J Biol Sci 12: 447-450.

62. Hosseinimehr SJ, Pourmorad F, Shahabimajd N, Shahrbandy K, Hosseinzadeh R (2007) In vitro antioxidant activity of Polygonium hyrcanicum, Centaurea depressa, Sambucus ebulus, Mentha spicata and Phytolacca americana. Pak J Biol Sci 10: 637-640.

63. Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B, et al. (2006) Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. Phytother Res 20: 961-965.

64. Gülçin I, Mshvildadze V, Gepdiremen A, Elias R (2006) The antioxidant activity of a triterpenoid glycoside isolated from the berries of Hedera colchica: 3-O-(beta-D-glucopyranosyl)-hederagenin. Phytother Res 20: 130-134.

65. Gülçin I, Mshvildadze V, Gepdiremen A, Elias R (2004) Antioxidant activity of saponins isolated from ivy: alpha-hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F. Planta Med 70: 561-563.

66. Amara-Mokrane YA, Lehucher-Michel MP, Balansard G, Duménil G, Botta A (1996) Protective effects of alpha-hederin, chlorophyllin and ascorbic acid towards the induction of micronuclei by doxorubicin in cultured human lymphocytes. Mutagenesis 11: 161-167.

67. Mendel M, Chłopecka M, Dziekan N, Wiechetek M (2011) The effect of the whole extract of common ivy (Hedera helix) leaves and selected active substances on the motoric activity of rat isolated stomach strips. J Ethnopharmacol 134: 796-802.

68. Dugo P, Donato P, Cacciola F, Germanò MP, Rapisarda A, et al. (2009) Characterization of the polyphenolic fraction of Morus alba leaves extracts by HPLC coupled to a hybrid IT-TOF MS system. J Sep Sci 32: 3627-3634.

69. Naowaratwattana W, De-Eknamkul W, De Mejia EG (2010) Phenolic-containing organic extracts of mulberry (*Morus alba* L.) leaves inhibit HepG2 hepatoma cells through G2/M phase arrest, induction of apoptosis, and inhibition of topoisomerase IIα activity. J Med Food 13: 1045-1056.

70. Pirvulescu MM, Gan AM, Stan D, Simion V, Calin M, et al. (2011) Curcumin and a Morus alba extract reduce pro-inflammatory effects of resistin in human endothelial cells. Phytother Res 25: 1737-1742.

71. Yang X, Yang L, Zheng H (2010) Hypolipidemic and antioxidant effects of mulberry (*Morus alba* L.) fruit in hyperlipidaemia rats. Food Chem Toxicol 48: 2374-2379.

72. Imran M, Khan H, Shah M, Khan R, Khan F (2010) Chemical composition and antioxidant activity of certain Morus species. J Zhejiang Univ Sci B 11: 973-980.

73. Loganayaki N, Manian S (2010) *In vitro* Antioxidant Properties of Indigenous Underutilized Fruits. Food Sci Biotechnol 19: 725-734.

74. Wei F, Yong FL, Ya LC, Dao NZ, Jin LR (2010) A new alkylene dihydrofuran glycoside with antioxidation activity from the root bark of *Morus alba* L. Chin Chem Lett 21: 821-823.

75. Chang LW, Juang LJ, Wang BS, Wang MY, Tai HM, et al. (2011) Antioxidant and antityrosinase activity of mulberry (*Morus alba* L.) twigs and root bark. Food Chem Toxicol 49: 785-790.

76. Singab AN, Ayoub NA, Ali EN, Mostafa NM (2010) Antioxidant and hepatoprotective activities of Egyptian moraceous plants against carbon tetrachloride-induced oxidative stress and liver damage in rats. Pharm Biol 48: 1255-1264.

77. Sivaci A, Sokmen M (2004) Seasonal changes in antioxidant activity, total phenolic and anthocyanin constituent of the stems of two Morus species (*Morus alba* L. and *Morus nigra* L.). Plant Growth Regul 44: 251-256.

78. Fischer A, Feller U (1994) Seasonal changes in the pattern of assimilatory enzymes and of proteolytic activities in leaves of juvenile ivy. Annals of Botany 74: 389-396.