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*RESEARCH ARTICLE*

# Evaluation of Antioxidant Activity of Various Herbal Folk Evaluation Medicine

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***Evaluation of Antioxidant Activity of Various Herbal Folk Medicine***

***Summary***

*This study was prepared to determine natural antioxidant capacity of some plants, which grow in Düzce and its surroundings, and are used as folk medicine. For this purpose, we investigated Smilax excelsa L., Mespilus germanica L., Equisetum telmateia Ehrh. , Urtica dioica L., and Laurocerasus officinalis Roemer plants and upper parts of the soil and leaves of these plants were extracted separately. In order to determine the antioxidant capacity of these plants, different in vitro methods such as N,N-dimethyl-p-phenylenediamine (DMPD·+) radical scavenging activity, cupric ion (Cu2+) reduction capacity, lipid peroxidation inhibiting activity were applied. Also phenolic contents of these plants were measured by Folin Ciocalteu's method. Vitamin C, gallic acid, and trolox were used as the reference antioxidant compounds. As a result of our study, we have found that while Smilax excelsa, Laurocerasus officinalis and Urtica dioica have relatively high content of phenolic compounds, Equisetum telmateia and Mespilus germanica have relatively low content of phenolic compounds. In general Smilax excelsa and Laurocerasus officinalis had higher antioxidant activity.*

***Key Words:*** *Antioxidant activity; phenolic content; folk medicine.*

***Çeşitli Bitkisel Halk İlaçlarının Antioksidan Aktivite Yönünden Değerlendirilmesi***

***Özet***

*Bu çalışma, Düzce ve çevresinde yetişen ve halk ilacı olarak kullanılan bazı bitkilerin total antioksidan kapasitesini belirlemek amacıyla yapılmıştır. Bu amaçla, Smilax excelsa L., Mespilus germanica L., Equisetum telmateia Ehrh., Urtica dioica L. ve Laurocerasus officinalis Roemer türleri incelenmiş olup, bitkilerin toprak üstü kısımları ve yaprakları ayrı ayrı ekstre edilmiştir. Bu bitkilerin antioksidan bileşiklerini ve antioksidan kapasitesini belirlemek için N,N-dimetil-p-fenilendiamin (DMPD·+) radikal giderme aktivitesi, kuprak metodu ile kuprik iyonları (Cu2+) indirgeme kapasitesi, tiyosiyanat metodu ile total antioksidan aktivite kapasite deneyleri çalışılmıştır. Ayrıca bitkilerin total fenolik içeriği Folin Ciocalteu metodu ile ölçülmüştür. Vitamin C, gallik asit ve troloks gibi bileşikler referans antioksidanlar olarak kullanılmıştır. Çalışmamızın sonucunda Smilax excelsa, Laurocerasus officinalis ve Urtica dioica nispeten daha yüksek fenolik madde içeriğine sahipken Equisetum telmateia ve Mespilus germanica daha düşük fenolik madde içeriğine sahip bulunmuştur. Genel olarak Smilax excelsa ve Laurocerasus officinalis türleri yüksek antioksidan aktivite göstermiştir.*

***Anahtar Kelimeler:*** *Antioksidan aktivite; fenolik madde içeriği; halk ilacı.*

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## INTRODUCTION

Oxygen, an element indispensable for life, can, under certain circumstances, adversely affect the human body. Oxidation processes are very important to living organisms (1). Reactive oxygen species (ROS) in the forms of superoxide anion ($\cdot O_2^-$), hydroxyl radical ($\cdot OH$), and hydrogen peroxide ($H_2O_2$) are generated in living organisms through many pathways (2).

When natural defences of the organism (enzymatic, non-enzymatic or dietary origin) are overwhelmed by an excessive generation of reactive oxygen and nitrogen species, a situation of oxidative stress occurs, in which cellular and extracellular macromolecules (proteins, lipids and nucleic acids) can suffer oxidative damage, causing tissue damage (3,4).

They are removed by antioxidant defense mechanisms. Antioxidants are considered to be possible protective agents, reducing oxidative damage from ROS in the human body and retarding the progress of many chronic diseases, as well as lipid peroxidation (5-8). There is a balance between the generation of ROS and antioxidant system in organisms (9).

Many antioxidant compounds, naturally occurring from plant sources, have been identified as free radical or active oxygen scavengers (10,11). Potential sources of antioxidant compounds have been searched in several types of plant materials such as: vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs (12).

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 ($OH^{\cdot}$) or superoxide radical ($O_2^{\cdot}$) scavengers (13,14).

Since ancient periods, herbs have been routinely used to treat wounds, and in many cultures their use in traditional medicine has persisted to the present (15). This study aims to determine antioxidant activities of *Smilax excelsa, Mespilus germanica, Equisetum telamateia, Urtica dioica,* and *Laurocerasus officinalis.* which are used as folk medicine in Düzce and its surroundings.

When a plant specimen is called by different vernacular names, information is classified according to their vernacular names (Table 1) (16).

## MATERIALS AND METHODS

### Chemicals

N,N-Dimethyl-p-phenylenediamine dihydrochloride (DMPD), linoleic acid, ammonium acetate were purchased from Fluka, ferric(III)chloride, **neocuproine** were obtained from Aldrich. Tween 20 was purchased from Sigma. Ethanol, sodium hydroxide (NaOH), hydrochloric acid (HCl), copper (II) chloride ($CuCl_2$) and potassium phosphate ($K_2HPO_4$) were obtained from Merck. Folin-Ciocalteu reagent, sodium carbonate ($Na_2CO_3$), copper(II) sulfate ($CuSO_4$), sodium potassium tartrate ($NaKC_4H_4O_6$) were purchased from Sigma-Aldrich.

### Plant material and extraction

Plant specimens of *Smilax excelsa, Mespilus germanica, Equisetum telmateia, Urtica dioica*, and *Laurocerasus officinalis* were collected from Düzce and its surroundings.

Plant leaves were extracted with 50% (w/v) ethanol and methanol EtAc and bidistilled water. A mass of 1 g of dry herbal matter was added to 15 ml of solvent, and was stirred up in a glass flask which had its mouth closed for 45 min. Than 5 ml of solvent was added and stirred up for 45 min; again. Finally 5 ml of solvent was added and stirred up for 15 min (totalling 105 min). First herbal extracts were filtered out of a fine textured cloth. Then extracts were filtered out using a filter paper. All studied plant extracts were portioned and stored at -80 ° C (17).

Antioxidant capacity assays were carried out with some modifications, using a microplate reader.

### Folin Ciocalteu

The amount of total soluble phenolics was determined according to the Folin-Ciocalteu method. Folin Ciocalteu's phenol reagent was diluted a volume

ratio of 1:3 with 96% EtOH prior to use. Lowry A solution was prepared from sodium carbonate such that the strength (w/v) of $Na_2CO_3$ in 0.1 M NaOH solution was 2 %.Lowry B solution wsa prepared from copper(II) sulfate such that the strength (w/v) of $CuSO_4$ in 1% sodium potassium tartrate ($NaKC_4H_4O_6$) solution was 0.5%. Lowry C solution was prepared by freshly mixing 50 ml Lowry A with 1 ml Lowry B.To 100 µl herbal extract was added 125 µl Lowry C. 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 to form blue colour. The absorbance against a reagent blank was measured at 750 nm (18).

**DMPD assay**

DMPD (100 mM) was prepared by dissolving 209 mg of DMPD in 10 ml of deionized 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 ($A_0$). 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 time, a decrease in absorbance was measured ($A_1$). Buffer was used as blank (19).

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

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

**CUPRAC assay**

$CuCl_2$ solution, $1.0\times10^{-2}$ M, was prepared by dissolving 0.17 g $CuCl_2{\cdot}2H_2O$ 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, $7.5\times10^{-3}$ M, was prepared daily by dissolving 0.039 g Nc in 96% ethanol. 50 µl $CuCl_2$ solution ($10^{-2}$ M), 50 µl neocuproine alcoholic solution ($7.5\times10^{-3}$ M) and 50 µl $NH_4Ac$ buffer solution, 27,5 µl sample and 27,5 µl water were added to wells. The mixture mixed well and incubate 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 (20).

**Thiocyante assay**

The reaction mixture consisted of 0.28 g linoleic acid, 0.28 g of Tween 20 and 50 ml of phosphate buffer

**Table 1.** General characteristics of the plants.

| Plant name | Local name | Part used | Use |
|---|---|---|---|
| Equisetum telmateia Ehrh. (Equisetaceae) | Dorukotu, Çamotu, At kuyruğu, Sazakotu, | Aerial parts of plant | To treat kidney stones and cardiovascular diseases. |
| Laurocerasus officinalis Roemer (Rosaceae) | Tahnal, Taflan, Karayemiş, Yaban yemiş | Leaf<br>Fruit | For stomach and kidney disorders, diabetes, haemorrhoids and as a panacea. |
| Mespilus germanica L. (Rosaceae) | Töngel, Döngel, Beşbıyık, Muşmula | Leaf<br>Fruit | To stop diarrhea. For cough, rheumatism, diabetes and haemorrhoids.<br>To treat inflammations. |
| Smilax excelsa L. (Liliaceae) | Melican, Meloncan Meravcan, Melovcan Burçman, Gıcırdikeni, Karasal Dikeni | Shoots<br>Root | For stomach aches and healing wounds. |
| Urtica dioica L. (Urticaceae) | Isırgan<br>Cibirgen<br>Cigirgen<br>Isırgan | Aerial parts of plant<br>Leaf<br>Seed | As panacea, for cancers, eczema, diabetes, hepatitis, haemorrhoids, rheumatic pain, healing wounds.<br>For gastrointestinal disorders. To treat kidney stones and infertility, as well as inflammation. |

(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 test sample was the control. After 24 h. 0.1 ml of the mixture was taken and mixed with 4.7 ml of 75% ethanol, 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 (21).

## RESULTS

### Folin Ciocalteu

The total phenolic contents of the plants were determined from regression equation of gallic acid calibration curve (y = 0,5358 ln(x)–1,2049)(Figure 1). The concentrations of total phenolic figure of extracts of plants are shown in (Table 2). The highest amount was found in the *table excelsa*, followed *Laurocerasus officinalis*, *Urtica dioica*, *Equisetum telmateia* and *Mespilus germanica.*

### DMPD assay

Different concentration of DMPD was carried out (figure 2) and trolox used as standard (figure 3). According to the data *Smilax excelsa* gave the highest antioxidant activity in methanolic extracts, *Laurocerasus officinalis* gave the highest antioxidant activity in aqueous extracts and *Mespilus germanica* gave the highest antioxidant activity in ethanolic extracts (figure 4,5 and 6).

**Table 2.** Total Phenolic Content of Plants

| AQUEOUS EXTRACT | Phenolic Content (µg/ml) |
|---|---|
| *Smilax excelsa* | 645,380777 |
| *Laurocerasus officinalis* | 618,2658113 |
| *Urtica dioica* | 477,0017788 |
| *Equisetum telmateia* | 171,8673141 |
| *Mespilus germanica* | 55,15913978 |

### CUPRAC assay

According to the results of the cupper reducing antioxidant capacity (CUPRAC) assay, the highest antioxidant capacities were observed for (figure 9) *Lurocerasus officinalis in* aqueous extracts, *Smilax excelsa in* methanolic extracts (figure 10) and *Equisetum telmateia* in ethanolic extracts (figure 11). Trolox and vitamin C were used as standard solutions (figure 7 and figure 8).

### Thiocyante assay

The antioxidant activities of plants on inhibition of linoleic acid peroxidation were assayed by thiocyanate method, are reported as gallic acid equivalents by reference to standard curve (figure 12). As shown in figure 13 *Smilax excelsa* gave the highest antioxidant activity and *Equisetum telmateia* gave the lowest antioxidant activity in aqueous extracts.

Folin Ciocalteu

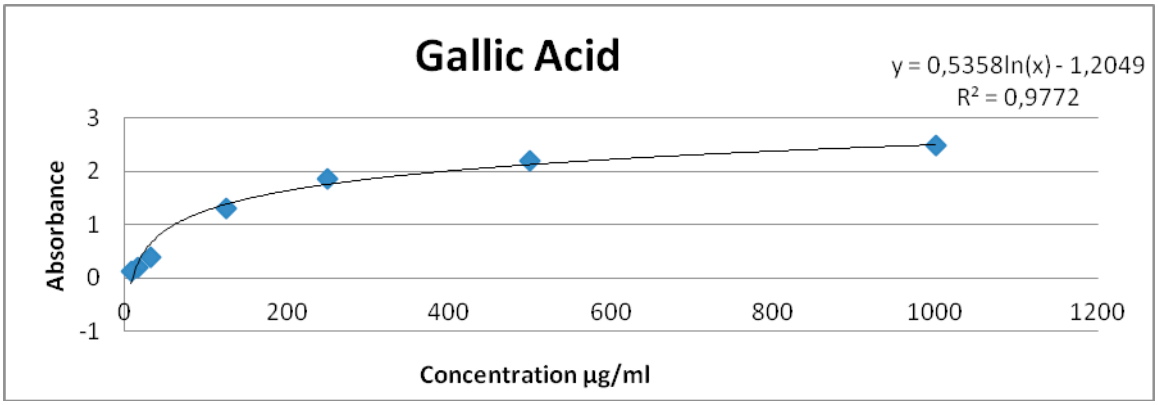


**Figure 1.** Standard curve of gallic acid.

**DMPD assay**

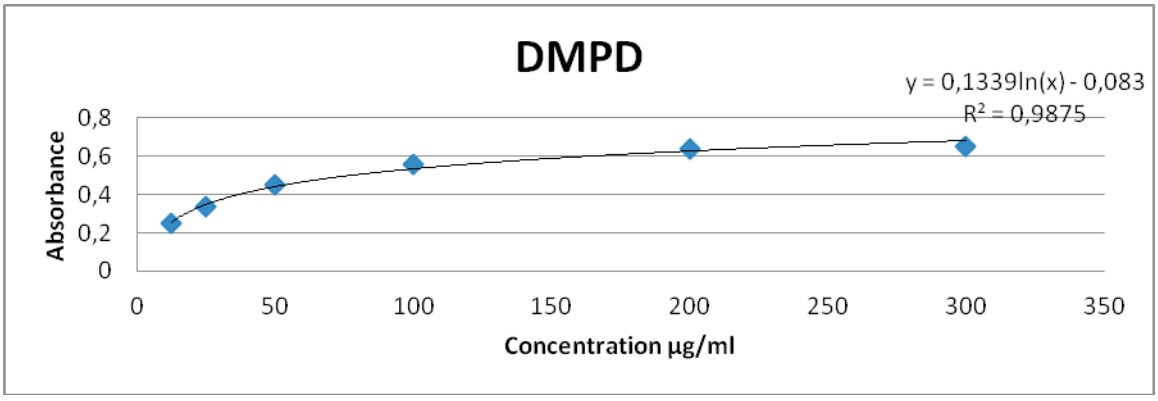


**Figure 2.** Relation of color formation to DMPD concentration.

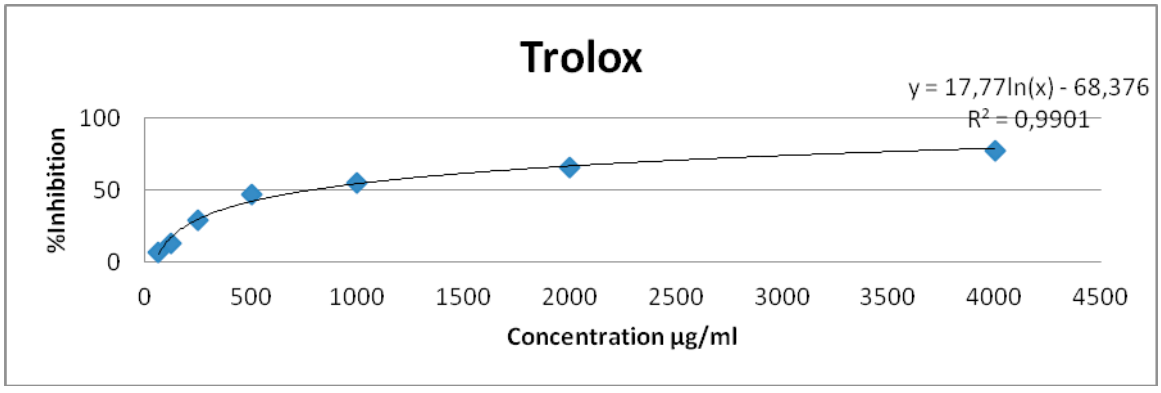


**Figure 3.** Scavenging of DMPD radical solution by Trolox

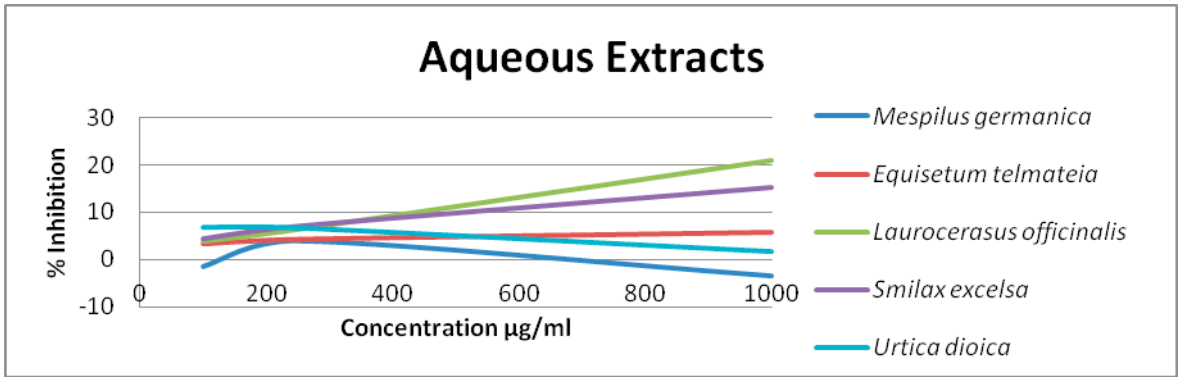


**Figure 4.** Scavenging of DMPD radical solution by aqueous extracts having different concentrations.

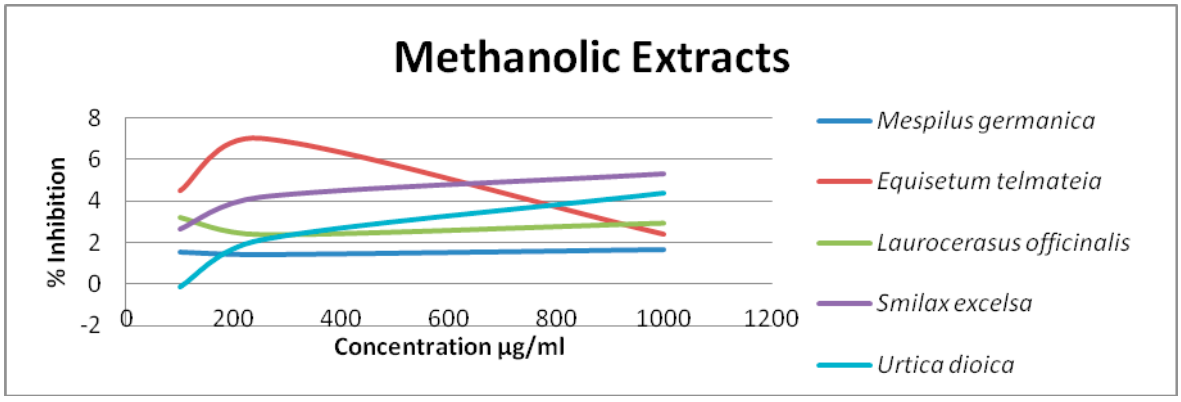


**Figure 5.** Scavenging of DMPD radical solution by methanolic extracts having concentrations.

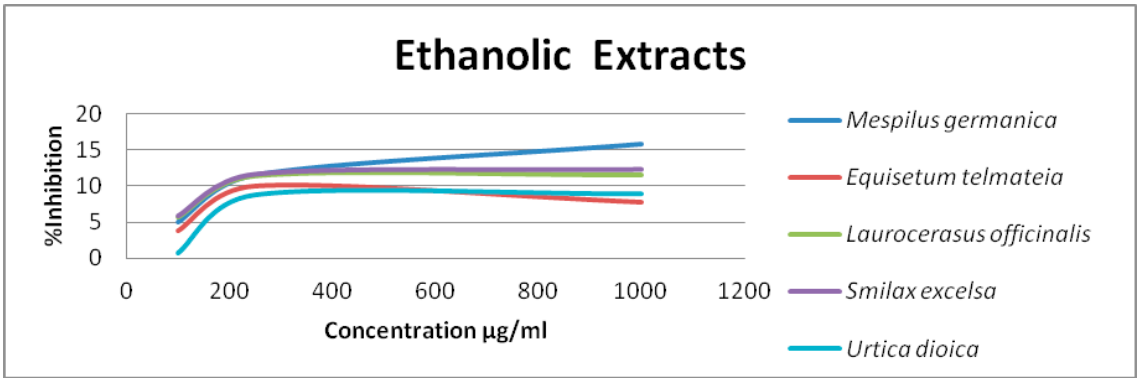


**Figure 6.** Scavenging of DMPD radical solution by ethanolic extracts having concentrations.

**CUPRAC**

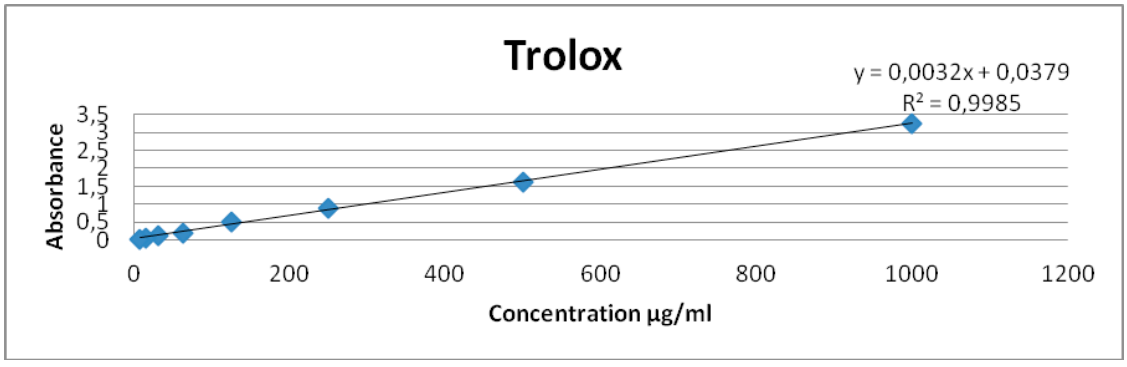


**Figure 7.** Standard Curve of Trolox.

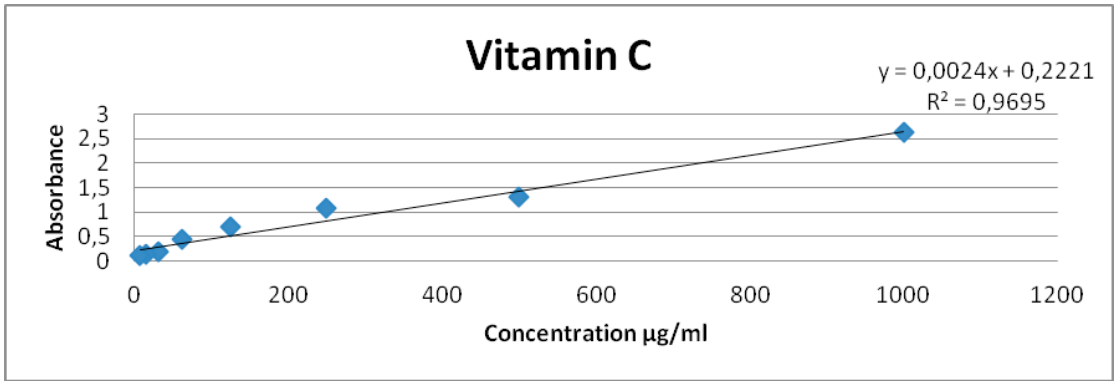


**Figure 8.** Standard Curve of Vitamin C.

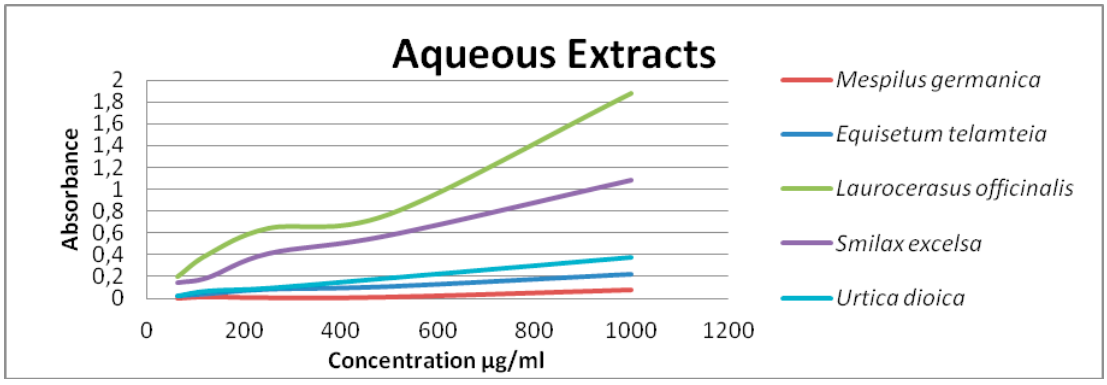


**Figure 9.** Antioxidant activities of aqueous plant extracts determined using CUPRAC method.

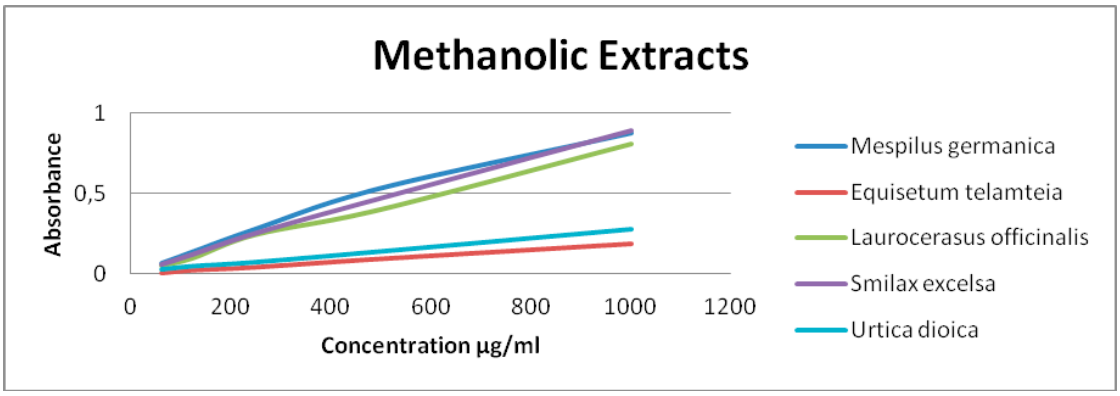


**Figure 10.** Antioxidant activities of methanolic plant extracts determined using CUPRAC method.

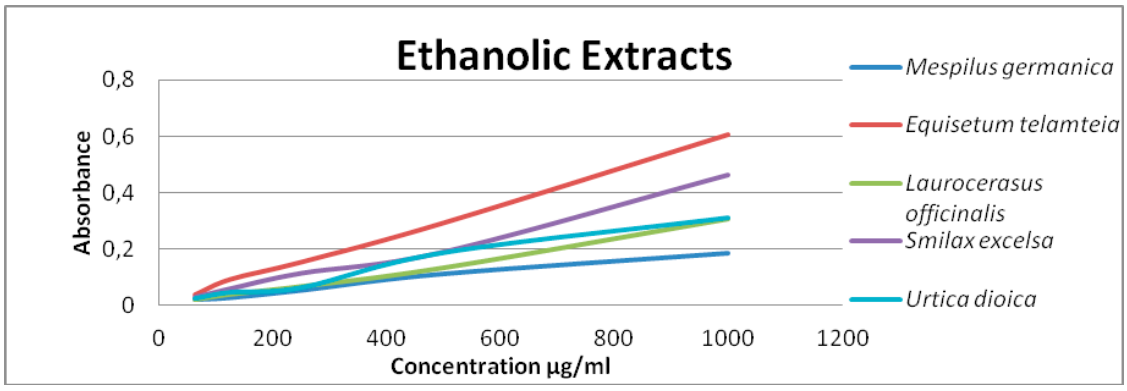


**Figure 11.** Antioxidant activities of ethanolic plant extracts determined using CUPRAC method.

### Thiocyante Assay

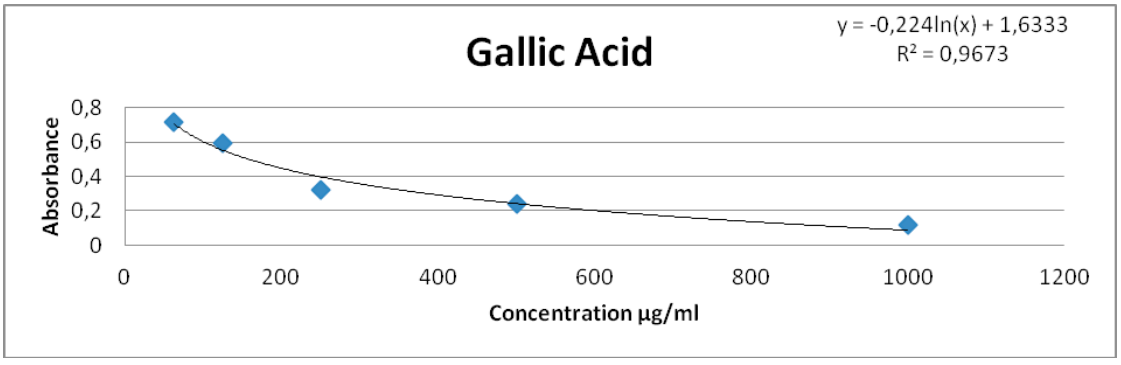


**Figure 12.** Standard curve of gallic acid

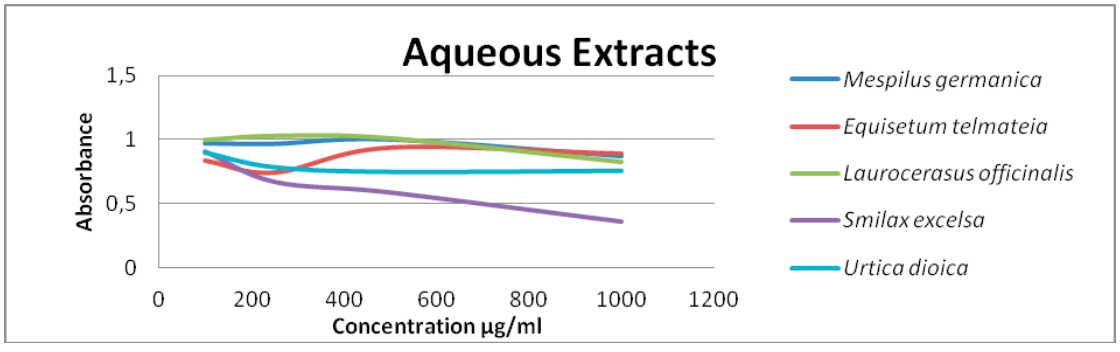


**Figure 13.** Antioxidant activities of aqueous plant extracts determined using the ferric thiocyanate assay

*excelsa in* methanolic extracts and *Equisetum telmateia* in ethanolic extracts. In general, on the basis of the results of this study, *Urtica dioica* has low antioxidant capacity in alcoholic extracts. These results were in contrast with another study (28) which was reported that *Urtica dioica* had powerful antioxidant activity against various oxidative systems in vitro. However, *Urtica dioica* compared with the other four plants in our study; the other study was carried out only *Urtica dioica* with aqueous extracts. The difference between two study might be due to the diversity of studied plants.

In DMPD method, the antioxidant capacity of the extracts was determined by inhibition of DMPD radical cation. While *Smilax excelsa and Laurocerasus officinalis* again gave the highest antioxidant activity in methanolic extracts and aqueous extracts, respectively, and *Mespilus germanica* gave the highest antioxidant activity in ethanolic extracts. From the DMPD results the percentage inhibition of DMPD radical cation of *Laurocerasus officinalis* were consistent with another study (29).

The ferric thiocyanate method was used to determine the amount of peroxide at the initial stage of lipid peroxidation (30). According to our results, the aqueous extract of Smilax excelsa has the highest inhibitory effect on the peroxidation of linoleic acid. A similar report found that the water extract of *Smilax excelsa* leaves strongly inhibited lipid peroxidation and showed radical scavenging (26). In contrast to our results most previous studies showed that *Urtica dioica* had good total antioxidant activity (28). Literature is scarce about reducing power in *Laurocerasus officinalis* and *Mespilus germanica* leaves. The outher study was generally based on *Laurocerasus officinalis* and *Mespilus germanica* fruit (30-32).

## CONCLUSION

In general, our study free radical scavenging and antioxidant activity capacity of plants related to their phenolic content. The results are consistent with each other and other antioxidant methods CUPRAC, DMPD and thiocyanate assay supported these results for aqueous extracts. In general, methanolic, ethanolic and aqueous extracts of *Smilax excelsa* have shown the highest antioxidant activity in CUPRAC, DMPD and thiocyanate assay. *Equisetum telmateia and Mespilus germanica* have relatively low phenolic content and antioxidant capacity. *Smilax excelsa*, *Lurocerasus officinalis* and *Urtica dioica* showed high antioxidant activity and have high phenolic content in aqueous extract.

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