ANTIOXIDANT ACTIVITY OF AQUEOUS AND ETHANOL EXTRACTS OF *CRATAEGUS MEYERI* POJARK LEAVES AND CONTENTS OF VITAMIN, TRACE ELEMENT

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ABSTRACT

The purpose of this study was to determine the antioxidant and antiradical activities in aqueous and ethanol extracts of *Crataegus meyeri* Pojark leaf, additionally, to examine some vitamins (A, E, C), trace elements (Cu, Zn, Mn, Se, Cr, Co). In this study, vitamin C, antioxidant and antiradical properties were determined using spectrophotometer. The results are compared with the reference antioxidants such as trolox, α-tocopherol and BHT. Levels of vitamin (A, E) measured using HPLC method. Trace elements were carried out using method of dry ashing with ICP-MS. The results of this study showed that *C. meyeri* leaf has a high antioxidant capacity and vitamin levels. *C. meyeri* is thought to be used as additives for food products and pharmaceutical industries with appropriate antioxidant properties and an antioxidant in future studies of experimental animal models, against free radicals generated in response to oxidative stress.

Key words: *Crataegus meyer*i Pojark, antioxidant, trace element, vitamin

INTRODUCTION

Natural foods and food derived antioxidants, such as vitamins and phenolic phytochemicals have received growing attention because they are known to function as chemopreventive agents against oxidative damage [1]. Reactive oxygen species, such as superoxide anion ($O_2^{\cdot-}$) radicals, hydroxyl radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) can cause oxidative damage to macromolecules, including DNA, proteins, lipids, and small cellular molecules. Free radicals have been implicated in the pathology of many diseases, including cancer, atherosclerosis, diabetes [2,3,4,5].

Antioxidants can be defined as compounds that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction. The antioxidant activity of phenolic compounds in plants is mainly due to their redox properties and chemical structure, which can play an important role in neutralizing free radicals, chelating transitional metals and quenching singlet and triplet oxygen, by delocalization or decomposing peroxides [6]. The antioxidant protection systems including enzymes (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymes protection (glutathione, vitamins C and E) play an important role in scavenging oxidants and preventing cell injury [5].

Medicinal plants have been used to treat human diseases in the world for centuries. People are more interested in medicinal plants because of their low toxicity and good therapeutic performance. Studies on antioxidant activity of medicinal plants have increased remarkably due to increased interest for their potential to be used as a rich natural source of antioxidants. Although to date, most research on the health benefits of plant-rich diets has focused on the established vitamins [7].

Vitamin A (retinol) and vitamin E (α-tocopherol) are lipid-soluble vitamins essential for human health. [8]. Vitamin C (ascorbic acid) is considered as a most important water-soluble antioxidant. It protects, compounds in extracellular and intracellular spaces in most biological systems and reduces tocopherol radicals back to their active form at the cellular membranes. It could directly scavenge superoxide radical, singlet oxygen, hydrogen peroxide, hydroxyl radical [9]. Trace elements of the transition metal group are known to activate enzymes or to be incorporated into metalloenzymes as electron transfer system (Cu, Fe, Mn and Zn) and also to catalyze valence changes in the substrate (Cu, Co, Fe and Mo). Some particular roles of several trace elements (Al, Cu, Co, Mo, Mn and Zn) which seem to be involved in protection mechanisms of frost-hardy and drought-resistance plant species are also documented [10].

Hawthorn (*Crataegus)* is a traditional medicinal plant. Consider a ‘‘cardiotonic’’ herb, the hawthorn plant has been used in traditional medicine to treat irregular heart beat, high blood pressure, chest pain, hardening of the arteries, and congestive heart failure; the antioxidants in hawthorn may help control high blood pressure and high cholesterol. The constituents responsible for the pharmacological effects of hawthorn preparations include flavonoids and oligomeric procyanidins [11].

The purposes of this work comprise the assessment of the antioxidant activity of leaf samples of *C. meyeri* ethanol and aqueous extracts by different analytical methods; total antioxidant activity**,** superoxide anion**,** DPPH, DMPD radical scavenging activity, reducing power, hydrogen peroxide scavenging**,** chelating effect and to determine their total phenolic and flavonoid contents, and to asses of the nutrient profile of these plant as due to their functional trace element (Cu, Zn, Mn, Se, Cr, Co) and vitamin (A, E, C) contents.

EXPERIMENTAL PROCEDURES

2.1. Plant material and extraction

C. meyeri leaf was collected in May-June in the stage of flowering in the Alacabuk Mountain, Bitlis, Yalınaqac village. A specimen was deposited in the Herbarium of the Department of Botany, Yuzuncu Yil University, Van F 13580. The plant sample was ground to fine powder using a Kenwood Multi-Mill (Kenwood Ltd., UK).

Extraction of *C. meyeri* leaves aqueous and ethanol was done according to the method of Gulcin et al.[12] with slight modifications. For aqueous extraction, 20 g *C. meyeri* were ground into a fine powder in a mill and was mixed with 400 mL boiling aqueous by magnetic stirrer for 15 minutes. For ethanol extraction, a 20 g *C. meyeri* sample was ground into a fine powder in a mill and mixed with 400 mL ethanol. The residue was re-extracted under the same condition until the extraction solvent became colorless. The combined extracts were filtered over whatman No. 1 paper. Extracts were evaporated to dryness in a vacuum by a rotary evaporator and crude ethanol and aqueous extracts were obtained by lyophilization with a freeze-drier. The crude extracts were placed in a dark bottle and stored at -20°C prior to further use.

2.2. Total antioxidant activity determination

The antioxidant activity of *C. meyeri* aqueous and ethanol extracts was determined according to the thiocyanate method of Mitsuda et al. [13]. 10 mg aqueous and ethanol extracts were dissolved in 10 mL water and ethanol. 10, 20 and 30 µg/mL of extracts or standards samples in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0) were added to linoleic acid 2.5 mL of emulsion in potassium phosphate buffer. 50 mL linoleic acid emulsion consists of 175 µg tween 20, 155 µL linoleic acid, and potassium phosphate buffer. The mixed solution was incubated at 37 ºC in a glass flask and in the dark. After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm in a spectrophotometer. The inhibition of lipid peroxidation in percentage was calculated by following equation:

$$\text{Inhibition (\%)} = \left\{ \frac{A_{Control} - A_{Sample}}{A_{Control}} \right\} X\ 100$$

Where $A_{control}$ was the absorbance of the control reaction and A_{sample} was the absorbance in the presence of the sample of *C. meyeri aqueous* and ethanol extracts [14].

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Various mechanisms, including reducing capacity, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging have been claimed to explain the antioxidant activities [15].

2.3. Reducing power

The reducing power of extracts was determined according to the method of Oyaizu[16] with slight modifications. Various concentrations of *C. meyeri* aqueous and ethanol extracts (15-45 μgmL^{-1}) in 1 mL of distilled water were mixed with 2.5 mL sodium phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 %). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of TCA (10 %) was added and centrifuged at 2500 rpm for 10 min. The upper layer of solution (2.5 mL 1 %) was mixed with distilled water (2.5 mL) and 0.5 mL ferric chloride ($FeCl_3$) (0.1 %), and the absorbance was measured at 700 nm against a blank. α-tocopherol and BHT were used as positive control. Increased absorbance of the reaction mixture indicated increased reducing power [4,17].

2.4. Hydrogen peroxide scavenging activity

The scavenging activity for hydrogen peroxide was measured according to the modified method of Ruch et al.[18]. Different concentrations of plant extract were added to 2 ml of H_2O_2 solution (10 mM) in phosphate buffer (50 mM, pH 7.4),and there action mixture was incubated at 25 °C for 30 min. The unreacted H_2O_2 was determined by measuring the absorbance of their action mixture at 230 nm with respect to the blank solution.

2.5. Superoxide anion radical scavenging activity

Superoxide radicals were generated by the method described by Zhishen et al. [19]. All solutions were prepared in a 0.05 M phosphate buffer (pH 7.8). Reaction was initiated by using a fluorescent lamp to illuminate the reaction mixtures containing the different concentrations of *C. meyeri* aqueous and ethanol extracts (15-60 μgmL^{-1}). The photo-induced reactions were performed using fluorescent lamps (20 W). The total volume of the reaction mixture was 3 mL. The reaction mixture was illuminated at 25 ºC for 40 min. The photochemically reduced riboflavin generated $O_2^{\cdot-}$ which reduced NBT to form blue formazan. The absorbance was measured at 560 nm. BHT and trolox were used as a positive control.

$$\text{Superoxide anion radical scavenging activity} \quad (\%) = \left\{1 - \frac{A_{sample}}{A_{control}}\right\} x100$$

2.6. Chelating activity on ferrous ions

The chelating effect was determined following the method of Dinis et al.[20]. Different concentrations of *C. meyeri* aqueous and ethanol extracts (10–30 µg/mL) in 0.4 mL methanol were added to a solution of 0.6 mM $FeCl_2$ (0.1 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Then, the mixture was shaken vigorously and left at room temperature for 10 minutes. Absorbance of the solution was then measured spectrophotometrically at 562 nm [12,20]. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated by using the formula given below:

$$\text{Metal chelating effect (\%)} = \left\{1 - \frac{A_{sample}}{A_{control}}\right\} x100$$

2.7. DMPD assay

The $DMPD^{\cdot+}$ radical scavenging activity of *C. meyeri aqueous* and ethanol extracts was determined according to the method described by Fogliano et al.[21]. Different concentrations of *C. meyeri* aqueous and ethanol extracts samples (10-30 μgmL^{-1}) were added to a spectrophotometric cuvette and total volumes of these samples were adjusted to 1 mL with distilled water. In the presence of an oxidant solution of iron chloride, DMPD, forms a colored radical cation, $DMPD^{\cdot+}$. The reaction is as follows: 1 ml of 100 mM DMPD is added to 100 ml of 0.1 M acetate buffer, pH 5.25, and the radical is obtained by adding 0.2 mL of 0.05 M ferric chloride. Scavenging assay proceeds by adding 0.1 mL of sample to 2 mL of radical solution and measuring the absorbance at 505 nm for 10 min after the addition of the sample. The percentage of $DMPD^{\cdot+}$ scavenging capability of *C. meyeri* aqueous and ethanol extracts was calculated using the following equation:

$$DMPD^{\cdot+} \text{ scavenging effect } (\%) = \left\{1 - \frac{A_{sample}}{A_{control}}\right\} x100$$

Where $A_{control}$ is the absorbance of the control, which contains 1 mL of control reaction and A_{Sample} is the absorbance in the presence of *C. meyeri* aqueous and ethanol extracts. $DMPD^{\cdot+}$ decreases significantly on exposure to radical scavengers [2,21,22].

2.8. Total phenolic assay

Total phenolic content in aqueous and ethanol extracts was measured using the modified folin–ciocalteu method. Singleton et al.[23]. 0.1 mL of the extract solution, containing 1000 µg extract was taken in a volumetric flask, 45 mL distilled water and 1 mL folin ciocalteu reagent was added and the flask was shaken thoroughly. After 3 min, 3 mL solution of Na_2CO_3 (2 %, w/v) was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm [24,25]. The same procedure was repeated with all standard gallic acid solutions (25–250 μgmL^{-1}). Phenolic contents are expressed as gallic acid equivalents per gram (mg GAE/g) of extract. The GAE values are expressed as means ± SEM of triplicate experiments.

2.9. Total flavonoids

The total flavonoid content was determined using aluminum chloride colorimetric method using quercetin as a standard and expressing the results as mg quercetin equivalents to the extract. Briefly, 1 mL of 2 % aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the methanolic extracts (2000 µg). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 1 mL extract solution with 1 mL methanol without $AlCl_3$ [26,27].

2.10. DPPH

DPPH assay the hydrogen atoms or electrons donation ability of the corresponding extract was measured from the bleaching of purple colored methanol solution of DPPH. This spectrophotometric assay uses stable radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH) as a reagent [28,29]. Fifty µL of various concentrations of the extract in methanol were added to 5 mL of a 0.004 % methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Antioxidant activity of the samples were expressed in term of IC_{50} values (μgmL^{-1} required to inhibit DPPH radical formation by 50 %), which was calculated form the graph plotted inhibition percentage against extract concentration. The IC_{50} values are expressed as means ± SEM of triplicate experiments. The synthetic antioxidant BHT was included in experiments as a positive control. Inhibition of free radical DPPH in percent was calculated by the following formula

$$\text{Inhibition (\%)} = \left\{\frac{A_{Blank} - A_{Sample}}{A_{Blank}}\right\} X\ 100$$

2.11. Determination of trace element contents

The collected samples were subsequently washed with double distilled water, cut, dried at 105 ºC for 24 h. Dried samples were homogenized and stored in pre-cleaned glass bottles until the analysis started. For the elemental (Cu, Zn, Mn, Se, Cr, Co) analysis, ICP-MS (Agilent 7500a USA) was used in this study.

C. meyeri samples were ground with a porcelain mortar and sieved (200 mesh). One gram of dry matter (as three parallel) was weighed in a porcelain crucible, followed by the addition of 2 mL of a mixture of ethyl alcohol and sulfuric acid (95:5) and burned. Ash was obtained at 500-550 ºC in a muffle furnace. Then the ash was dissolved with 4 mL 3 N HCl and the solution transferred to a 50 mL calibrated flask and diluted to 50 mL with double distilled water and filtered after 5 h with blue-band filter paper and regulated to 50 mL.

2.12. Vitamin (A, E) analysis

2.12.1. Standard solutions and calibration

α-tocopherol and all-trans-retinol stock solutions were prepared at 500 μgmL^{-1} in methanol. Stock solutions were appropriately diluted with the mobile phase for standard solution preparation. Calibration was calculated by linear regression analysis of the peak area versus the standard solution concentrations.

2.12.2. Extraction procedure

5 gr of *C. meyeri* was extracted by hexane and ethanol (containing 0.01 %

BHT), and the sample was vortexed for 1 min. [30]. The sample was extracted for 24 h in dark. The sample was vortexed and centrifuged at 7000 rev/min for 10 min. Supernatant was filtered using whatman filter paper, and 500 µL of hexane layer was extracted and evaporated to dryness under a stream of nitrogen at 37 ºC. The residue was dissolved in THF (50 µL) and methanol was added (150 µL). The sample was vortexed for 1 min. then, 100 µL samples were autosampled using amber glass vials.

2.12.3. Chromatographic conditions

The chromatographic system consisted of a Thermo Scientific Finnigan Surveyor with a photodiode array (PDA) detector, and a tray autosampler (-8 °C). The data were processed using Thermo Scientific ChromQuest version 4.2 software. Separation was performed with a 5 µm Gl Science C_{18} reverse-phase column (250 x 4.6 mm ID). The mobile phase of a methanol-THF mixture (80:20, v/v) was modified from [8,31,32]. Pump was set at a flow rate of 1.5 mL/min. The chromatogram was monitored with photodiode array detector (PDA) array detection at 325 and 290 nm (α-tocopherol and all-trans-retinol, respectively). The chromatographic analysis was carried out at 40 ºC with isocratic elution.

2.13. Vitamin C analysis

Vitamin C stock solution was prepared at 4000 mg/ ml in metaphosphoric acid. Stock solutions were appropriately diluted with double-distilled water for standard solution preparation. Calibration was calculated by linear regression analysis of the absorbance versus standard solution concentrations. Vitamin C content of *C. meyeri* leaf was measured spectrophotometrically (Shimadzu UV 1800, Japan) at 521 nm according to 2,4-dinitrophenyl hydrazine (DNPH) method [33].

2.14. Statistical analysis

The results are expressed as mean values and standard errors of mean ($\overline{X}$ ± SEM). The statistical analysis was carried out with One-way analysis of variance (ANOVA) using a statistical package program (SPSS 22.0 for Windows). Samples were carried out in triplicate. Nonlinear regression analysis was used to calculate the IC_{50} values.

RESULTS

The present study evaluated in vitro antioxidant activities of *C. meyeri* Pojark leaves ethanolic and aqueous extract, in terms of total antioxidant activity, DPPH radical scavenging activity, DMPD free radical scavenging activity, superoxide anion radical scavenging activity, hydrogen peroxide scavenging activity, metal chelating activity, total reducing power, total phenolic and flavonoid vitamin and trace elements contents.

Trace element and vitamin content of *C. meyeri* leaves is presented in Table 1. The contents of copper, zinc, manganese, selenium, chromium and Cobalt in *C. meyeri* leaves were found 21,99 ± 2,01, 47,32 ± 2,44, 51,01 ± 2,26, 0,091 ± 0,01, 1,72 ± 0,09 and 1,18 ± 0,05 µg g^{-1}, the contents of α-tocopherol, retinol and ascorbic acid in *C. meyeri* leaves were found 2,55 ± 0,19, 0,41± 0,024 and 202,05 ± 12,46 µg g^{-1} respectively on dry weight basis.

Table 1: Level of Vitamin (E,A,C) and trace element (Cu, Zn, Mn, Se, Cr, and Co) in *Crataegus meyeri* Pojark leaves samples.

Parameters	*Crataegus meyeri* Pojark (Rosaceae)
	$\overline{X}$ ± SEM
α-tocopherol (µg g^{-1} dw)	2,55 ± 0,19
Retinol (µg g^{-1} dw)	0,41± 0,024
Vitamin C (mg 100 g^{-1} dw)	202,05 ± 12,46
Cu (µg g^{-1} dw)	21,99 ± 2,01
Zn (µg g^{-1} dw)	47,32 ± 2,44
Mn (µg g^{-1} dw)	51,01 ± 2,26
Se (µg g^{-1} dw)	0,091 ± 0,01
Cr (µg g^{-1} dw)	1,72 ± 0,09
Co (µg g^{-1} dw)	1,18 ± 0,05

All values are mean of triplicate determinations expressed on dry weight basis. This is the first work on vitamin and trace element in *C. meyeri* leaves, so we could not compare data with earlier workers data.

Table 2 shows absorbance values of reducing power different concentrations of *Crataegus meyeri* Pojark extracts, α-tocopherol and BHT. Table 3 shows antioxidant activity of aqueous and ethanol extracts of *C. meyeri*. Total antioxidant activity was determined by the thiocyanate method. Both *C. meyeri* leaf extracts exhibited effective and powerful antioxidant activity at all concentrations.

Figure 1 illustrated antioxidant activity of different concentrations (from 10 mg/mL to 30 mg/mL) of aqueous extract of *C. meyeri* leaves; BHT, α-tocopherol and trolox end of 48th hour % inhibition values in the linoleic acid emulsion was determined by the thiocyanate method.

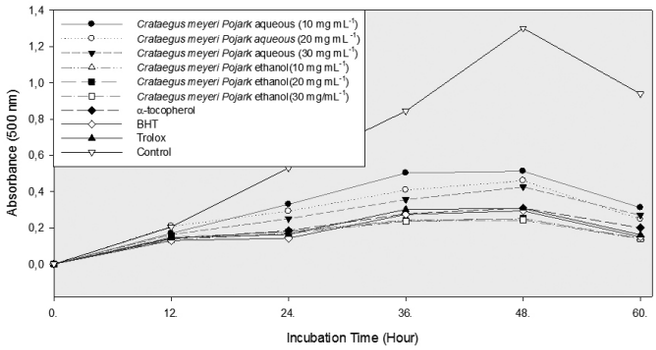

Fig 1: Antioxidant activity of different concentrations of aqueous and ethanol extract of *Crataegus meyeri* Pojark , as compared to positive controls BHT, α-tocopherol and trolox in the linoleic acid emulsion was determined by the thiocyanate method.

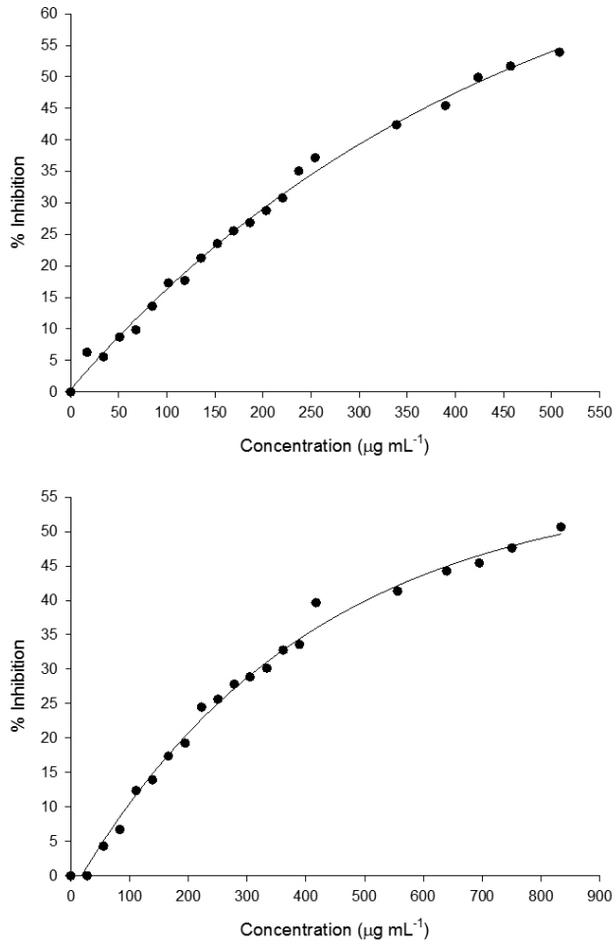

Fig. 2: Inhibition of DPPH radical versus concentrations of *Crataegus meyeri* Pojark ethanol and aqueous extract.

The results concerning the radical scavenging activity of different extracts *C. meyeri*, along with the standard reference BHT, are shown in table 3. The best free radical scavenging activity was obtained with the ethanolic extract (IC_{50} 435,13 ± 2,71 μg mL^{-1}), while the aqueous extract showed comparable levels of free radical scavenging activity with an IC_{50} value of 877,73 ± 6,98 μg mL^{-1}. The reducing power of the aqueous and ethanol extract of *C. meyeri* leaves increased with concentration. At 45 μg mL^{-1}, the reducing power was higher for the all extracts. According to the results, the most active extract of *C. meyeri* leaves was aqueous with an absorbance value of 0,2110 ± 0,001. At this concentration value were found reducing power of α-tocopherol 0,6093 ± 0,002, BHT 0,6090 ± 0,003 and ethanol extract 0,1840 ± 0,001 (Table 2)

Figure 3 shows the reductive capabilities of *C. meyeri* leaves extracts compared to BHT, and α-tocopherol. For the measurements of the reductive ability, it was determined absorbance values of reducing Power of different concentrations (15-45 μg mL^{-1}).

Table 2: Absorbance values of reducing power different concentrations of *Crataegus meyeri* Pojark extracts, α-tocopherol and BHT.

Concentration	Abs. ($\overline{X}$ ± SEM)
C. meyeri Pojark aqueous ext. (15 μg mL^{-1})	0,1427 ± 0,001
C. meyeri Pojark aqueous ext. (30 μg mL^{-1})	0,1773 ± 0,002
C. meyeri Pojark aqueous ext. (45 μg mL^{-1})	0,2110 ± 0,001^{a,a1}
C. meyeri Pojark ethanol ext. (15 μg mL^{-1})	0,1283 ± 0,001
C. meyeri Pojark ethanol ext. (30 μg mL^{-1})	0,1480 ± 0,001
C. meyeri Pojark ethanol ext. (45 μg mL^{-1})	0,1840 ± 0,001^{a2,a3}
α-tocopherol (15 μg mL^{-1})	0,2427 ± 0,002
α-tocopherol (30 μg mL^{-1})	0,4283 ± 0,001
α-tocopherol (45 μg mL^{-1})	0,6093 ± 0,002^{a,a2}
BHT (15 μg mL^{-1})	0,2600 ± 0,011
BHT (30 μg mL^{-1})	0,4493 ± 0,003
BHT (45 μg mL^{-1})	0,6090 ± 0,003^{a1,a3}
Control	0,1520 ± 0,011

a,a1,a2,a3: $p<0.001$ (different letters, significant differences between groups)

As seen in figure 3, $DMPD^{\bullet+}$ free radical scavenging activity of *C. meyeri* ethanol and aqueous extracts was determined different concentrations (15-45 μg mL^{-1}) as compared to positive controls BHT and α-tochopherol. Both plant extracts demonstrated high $DMPD^{\bullet+}$ scavenging activity. As a result of the statistical analysis (Table 3), it was found that the level of $DMPD^{\bullet+}$ radical scavenging activity **(**%) in *C. meyeri* aqueous decreased according to α-tochopherol ($p<0.05$), In addition, *C. meyeri* ethanol was significantly higher BHT ($p<0.001$).

The effects of the leaf extracts obtained from *C. meyeri* on the superoxide anions generated by NBT were determined and are shown in Table 3. The different concentrations of *C. meyeri* in the leaf extracts showed antioxidant activities in 60 μg mL^{-1} concentration in the superoxide anion scavenging assay. Superoxide anion scavenging activities of the *C. meyeri* leaf extracts (aqueous, ethanol), BHT, trolox accounted for an inhibition of 42,74 ± 2,88 %, 50,85 ± 2,21 % 18,49 ± 4,25 % and 52,65 ± 1,59 %, respectively,

The results are shown in Table 3. The total extract and ethanol fraction exhibited a strong effect, comparable to that of BHT and trolox, used as a positive control. The best superoxide scavenging activity was shown by the ethanol fraction, followed by the aqueous extract. These preparations inhibited the development of the color, produced during the reaction of superoxide with NBT by 50,85 ± 2,21 and 42,74 ± 2,88 % respectively. The values obtained were similar to that of trolox (52,65 ± 1,59 % inhibition). The results showed that the aqueous and ethanol extracts had antioxidative potential, similar to positive control (trolox).

Regarding hydrogen peroxide scavenging activity (%), it was seemed that *C. meyeri* aqueous levels compared with the BHT decreased ($p<0.01$) and It was observed that the level *C. meyeri* ethanol decreased according to BHT and trolox ($p<0.001$), ($p<0.01$) respectively (Table 3)

The ferrous ion chelating activities of *C. meyeri* ethanol and aqueous extracts, BHT, α-tocopherol, and trolox different concentration (10-30 μg mL^{-1}) are shown in Fig. 3.

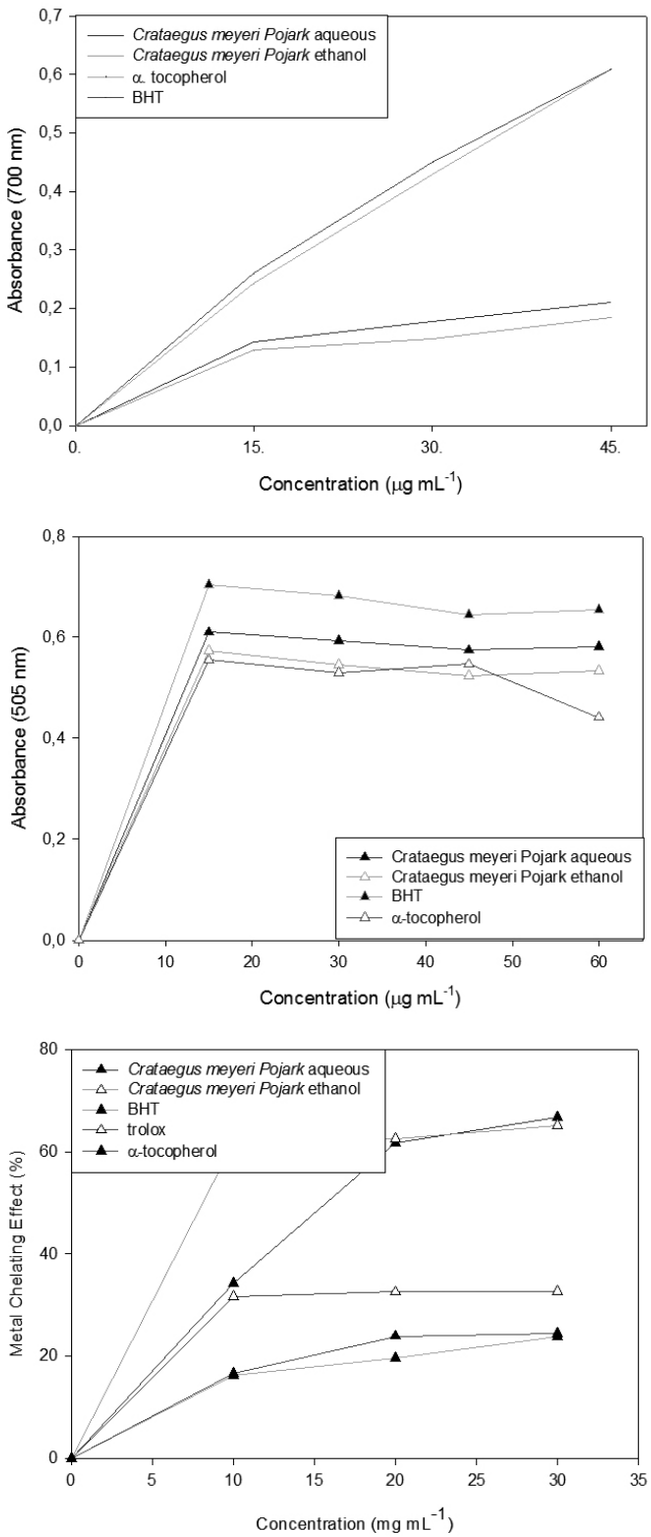

Fig. 3: Absorbance values of reducing Power of different concentrations (15-45 μg mL^{-1}), $DMPD^{\bullet+}$ free radical scavenging activity (15-45 μg mL^{-1}) and metal chelating effect of (10-30 μg mL^{-1}) of aqueous and ethanol extracts of *Crataegus meyeri* Pojark, as compared to positive controls.

Table 3: The percent values of total antioxidan activity, $DPPH^{\bullet+}$, superoxide anion radical, hydrogen peroxide, $DMPD^{\bullet+}$ radical scavenging activities and metal chelating effect different extracts of *Crataegus meyeri* Pojark leaves as compared to positive controls.

Concentration (48.hour)	**Total antioxidant activity % ($\bar{X}$ ± SEM)**
C. meyeri Pojark aqueous ext. (10 µg mL^{-1})	68,42 ± 4,61 [c, c1, c2]
C. meyeri Pojark aqueous ext. (20 µg mL^{-1})	71,45 ± 5,88
C. meyeri Pojark aqueous ext. (30 µg mL^{-1})	74,27 ± 4,37
C. meyeri Pojark ethanol ext. (10 µg mL^{-1})	84,67 ± 1,11 [c]
C. meyeri Pojark ethanol ext. (20 µg mL^{-1})	84,63 ± 0,85 [c1]
C. meyeri Pojark ethanol ext.. (30 µg mL^{-1})	85,22 ± 0,43 [c2]
α-tocopherol (30 µg mL^{-1})	81,08 ± 0,73
BHT (30 µg mL^{-1})	82,09 ± 0,39
Trolox (30 µg mL^{-1})	81,23 ± 1,05
Plant ext.	**DPPH scavenging activities %**
C. meyeri Pojark aqueous ext.	50,59 ± 0,19
C. meyeri Pojark ethanol ext.	53,84 ± 0,09
BHT	52,95 ± 0,41
Concentration (45 µg mL^{-1})	**$DMPD^{\bullet+}$ radical scavenging activity %**
C. meyeri Pojark aqueous	45,83 ± 0,2257 [c]
C. meyeri Pojark ethanol	50,61 ± 0,8034 [a]
BHT	39,17 ± 0,2397 [a]
α- tochopherol	48,41 ± 0,4599 [c]
Concentration (60 µg mL^{-1})	**Superoxide anion radical scavenging activity %**
C. meyeri Pojark aqueous	42,74 ± 2,88 [b]
C. meyeri Pojark ethanol	50,85 ± 2,21 [a]
BHT	18,49 ± 4,25 [b,a,]
Trolox	52,65 ± 1,59
Concentration (60 µg mL^{-1})	**Hydrogen peroxide scavenging activity %**
C. meyeri Pojark aqueous	48,14 ± 0,8652 [b]
C. meyeri Pojark ethanol	53,37 ± 1,008 [a,b1]
BHT	40,93 ± 0,7256 [b,a]
Trolox	46,98 ± 1,141 [b1]
Concentration (30 µg mL^{-1})	**Metal chelating effect %**
C. meyeri Pojark aqueous	54,23 ± 10,07 [b,b1]
C. meyeri Pojark ethanol	63,16 ± 1,033 [a,b2,a1]
BHT	19,93 ± 2,165 [b, a]
trolox	32,33 ± 0,3307 [b2]
α-tocopherol	21,68 ± 2,501 [b1,a1]

a,a1: p<0.001, **b, b1,b2**: p<0.01, **c,c1,c2**: p<0.05 (different letters, significant differences between groups)

The total phenolics of various extract of plant were measured using folin–ciocalteau's assay while the total flavonoids were estimated using aluminum chloride method for flavonoids. Total phenolic and flavonoid contents of plant extract were determined and expressed in mg of gallic acid and quarcetin equivalents, respectively.

Absorbance (λ_{760nm}) = = 0,0011 x [GAE(mg)] + 0,0747 (r^2: 0,9930)

Total phenolic and flavonoid contents the present study was carried out to evaluate the total phenolic and flavonoid contents of the *C. meyeri* aqueous and ethanol extracts. Total phenolic content are commonly found in plant have been reported to have several biological activities including antioxidant activity. The total phenolic contents of the extracts from the *C. meyeri* aqueous and ethanol were 9,056 ± 0,35 mg GA g^{-1} and 11,291 ± 0,021 mg GA g^{-1}, respectively. Flavonoid contents of the extracts from the *C. meyeri* aqueous and ethanolic extracts were 2,15 ± 0,008 mg QE g^{-1} and 3,56 ± 0,012 mg QE g^{-1}, respectively.

Absorbance (λ_{415nm}) = 0,012 x [QE (mg)] -0,0149 (r^2: 0,9962)

DISCUSSION

Reactive oxygen species such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous factors and exogenously result in extensive oxidative damage that in turn lead to geriatric degenerative conditions, cancer and a wide range of other human diseases. Carotenoids, the natural pigments from plant origin react rapidly with these free radicals and retard or alleviate the extent of oxidative deterioration [17].

Current results of total antioxidant activity indicate that the inhibition (%) of *C. meyeri* aqueous ext. (10 μg mL^{-1}) was significantly lower than ethanol ext. (10, 20, 30 μg mL^{-1}) ($p<0.05$), ($p<0.05$), ($p<0.05$) respectively. *C. meyeri* ethanol extract showed a high antioxidant activity. Aqueous extract scavenged less radical than ethanol extract.

The DPPH free radical scavenging activity is a widely used model for evaluating the free radical scavenging ability of various compounds. The absorbance decreased at 517 nm, resulting in a color change from purple to yellow, as radicals were scavenged by antioxidants through the donation of hydrogen to form the stable DPPH molecule. The concentration of sample necessary to reduce the initial concentration of DPPH by 50 % (IC_{50}) under the experimental conditions was determined. A lower value if IC_{50} indicates higher antioxidant activity [3]. The concentration of the sample necessary to reduce the initial concentration of DPPH by 50 % (IC_{50}) under the experimental conditions was determined. A lower value of IC_{50} indicates higher antioxidant activity. The best free radical scavenging activity was obtained with the ethanolic extract (IC_{50} 435,13 ± 2,71 μg mL^{-1}), while the aqueous extract showed comparable levels of free radical scavenging activity with an IC_{50} value of 877,73 ± 6,98 μg mL^{-1}. It can be concluded that the ethanolic extract of the *C. meyeri* (Fig 2) showed more potent in vitro antioxidant activity, with higher percentage inhibition, than the aqueous extract of the *C. meyeri* (Fig.2).

Barreira et al., [34] has a reported that *Crataegus monogyna* flower ethanolic and aqueous extract were found 167 ± 6,0 μg mL^{-1} 811 ± 14 μg mL^{-1} respectively. In this study was obtained *C. meyeri* leaves ethanolic extract (IC_{50} 435,13 ± 2,71 μg mL^{-1}), while the aqueous extract showed comparable levels of free radical scavenging activity with an IC_{50} value of 877,73 ± 6,98 μg mL^{-1}. It was seen in our study that DPPH scavenging activity (%), *C. meyeri* leaves ethanolic and aqueous extract were determined % 53,84 ± 0,09 and % 50,59 ± 0,19, however Liu et al., [35] found % 55,34 ± 0,85 in hawthorn fruit.

Reducing power, which was used to measure the reductive ability of antioxidants, was evaluated by the transformation of Fe (III) to Fe (II) in the presence of aqueous and ethanol extract of *C. meyeri* leaves. Antioxidants reduce the Fe^{3+} ferricyanide complex to the ferrous form by donating an electron. The color of the test solution then changes from yellow to different shades of green and blue [3]. The absorbance of *C. meyeri* aqueous ext. was significantly lower than positive controls α-tocopherol and BHT ($p<0.001$), ($p<0.001$). On the other hand, *C. meyeri* ethanol ext. was significantly lower than those positive controls α-tocopherol and BHT ($p<0.001$), ($p<0.001$) respectively.

Generally, superoxide anions convert to oxygen and hydrogen peroxide by superoxide dismutase, or they react with nitric oxide to form peroxynitrite. Hydrogen peroxide can be converted into water and oxygen by catalase. Therefore, superoxide scavenging capacity in the human body is very important as the first line of protection against oxidative stress [1]. The superoxide anion radical scavenging activities (%) of all of the plant extracts were significantly higher than those of BHT ($p<0.01$), ($p<0.001$). The superoxide anion scavenging activity of the *C. meyeri* aqueous extracts showed no significant difference compared to the *C. meyeri* ethanol extracts (Table 3).

Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via fenton chemistry. The processes can be delayed by iron chelation and deactivation. Therefore, the ability of the extracts to chelate iron (II) ions was evaluated and expressed as % chelation capacity [7]. The results show that in metal chelating effect (%) *C. meyeri* aqueous level were higher than BHT and α-tocopherol ($p<0.01$), ($p<0.01$) respectively. On other hand, *C. meyeri* ethanol level was significantly higher BHT, trolox and α-tocopherol ($p<0.001$), ($p<0.01$), ($p<0.001$) respectively (Table 3).

Phenolic content of aqueous extract of *C. meyeri* to compared ethanolic extract was significantly different ($P<0.001$), On the other hand, total flavanoid content of *C. meyeri* aqueous extract was significantly lower than ethanolic extract ($P<0.01$). The total phenolic and flavonoid contents of the *C. meyeri* leaves ethanolic extract was higher than content of aqueous extract, these results suggest that the higher levels of antioxidant activity were due to the presence of phenolic and flavonoid components.

Phenolic and flavonoid compounds may be responsible for the free radical scavenging activity that was observed. Total phenolic concentration, expressed as gallic acid equivalents showed correlation with the antioxidant activity. In this study, we found that total phenolic and flavonoid contents of the *C. meyeri* leaves aqueous and ethanolic extract were comparable with the levels found by the researchers [35,36]. and also found that the mean total phenolic levels of ethanol and aqueous extract were found 9,056 ± 0,35 mg GA g^{-1} and 11,291 ± 0,021 mg GA g^{-1}, total flavanoid levels of ethanol and aqueous extract 2,15 ± 0,008 mg QE g^{-1} and 3,56 ± 0,012 mg QE g^{-1}. These results of leaves extract were lower than [35] who determined the total phenolic (32,75 ± 1,20 mg GA g^{-1}) and flavonoid (50,97 ± 2,24 mg RE g^{-1}) contents in hawthorn fruit, in other study [36] reported that *Crataegus azarolus* leaves methanolic and aqueous extract were found the total phenolic contents 30,6 ± 2,5 mg GA g^{-1} and 13,6 ± 1,8 mg GA g^{-1}respectively.

Ethanol extract of *C. meyeri* has higher total antioxidant activity, DPPH radical scavenging activity, DMPD, free radical scavenging activity, superoxide anion radical scavenging activity, hydrogen peroxide scavenging activity, metal chelating effect, total phenolic and flavonoid contents than its aqueous extract. In contrast, total reducing power of ethanolic extract was lower than aqueous extract. *C. meyeri* aqueous extract had good reducing power. Therefore, ethanolic extract of *C. meyeri* can be more effective antioxidant than its aqueous extract.

All the extracts proved to have free radical-scavenging activity and reducing power, but to different extent. The antioxidant activity was measured against radical species generated in their action system, such as DPPH radicals (DPPH scavenging activity assay) or by the reducing effect on Fe^{3+}/ferricyanide complex (reducing power assay). Ethanolic extracts gave higher antioxidant activity (lower EC_{50} values; than aqueous extracts, which is in agreement with the highest content in phenolics found in the ethanolic extracts.

CONCLUSIONS

C. meyeri the leaf extracts exhibited superior and potent antioxidant capacities in terms of the total antioxidant activity, DPPH radical scavenging activity, DMPD free radical scavenging activity, superoxide anion radical scavenging activity, hydrogen peroxide scavenging activity, metal chelating effect assays, and these results were in consistent with high total phenolic, flavonoid and vitamin (A, E, C) contents values. The present study demonstrates high phenolic, flavonoid content and antioxidant potential of *C. meyeri* leaves ethanolic and aqueous extract that could contribute to sustain antioxidant status and protect against free radical damage. *C. meyeri* is thought to be used as additives for food products and pharmaceutical industries with appropriate antioxidant properties and an antioxidant in future studies of experimental animal models, against free radicals generated in response to oxidative stress; also, it is thought that these data will be reference for future studies.

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