The Determination of Antioxidant Vitamin Levels, Radical Scavenging Effect of Elaeagnus Angustifolia Methanol Extracts Against to MDA

Research Article

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ABSTRACT

Free radical; atomic or molecular structures with single-electron compounds. With other molecules that can be very easy to electron exchange across the molecules "an oxidant molecules" or "reactive oxygen particles" also be said. Antioxidants compound that stop the formation of the free radicals or dispel negative effects of items. this, to do inhibited the radical reaction systems. or non-toxic products there is no turning it on. The most of antioxidants found in foods. With an antioxidant-rich diet, free radicals and active oxygen consisting of the which is the basis of disease oxidative damage can be prevented [1]. This the nutrition-type especially fruits vegetables natural anti-oxidant resources [2]. In this study aimed that to determine the Antioxidant vitamin levels, radical-scavenging properties of *Elaeagnus angustifolia* methanol extracts against to prevent formation MDA. The material obtained from a plant seller in Elazığ. Extracted from plants was performed using methanol solvent. Extraction process was done of plant parts by weight of 2 grams in weight was 10 times the solvents. Spectral analysis with Shimadzu model UV 1400 spectrophotometer, antioxidant vitamins with the same brand HPLC was carried out. Plant extracts of the DPPH radical knockdown activity the method specified,[3] the OH-radical destruction the method indicated[4], Antioxidant capacity ABTS radical destruction activity[5] lipid peroxidation (LPO) measurements using as standard Fenton Quercetin, Resvesterol reagents MDA-TBARS by the method[6], vitamins analysis using the methods [7, 8, 9] was performed. Radical scavenging activity from 100μL-800μL concentration measurements were performed with two evil-fold increase. Results of DPPH radical scavenging and $OH^{\cdot}$ radical knock down activity increased with concentration difference. MDA analysis comparison with the control group showed significant differences. MDA analysis with controls Quercetin standards compared were not statistical different, both the extracted measurements and control groups fenton reagent and resveratrol standard compared with a significant statistical difference was observed. $p<0,05$. Vitamin K1 was found at the lowest level. Vitamin E and vitamin K2 was detected similar pattern. Vitamin D2, vitamin E, and vitamin K2 almost 4 times more than, approximately 8 times greater than vitamin K1 found. Vitamin A level in fat soluble all vitamins the highest level of detected. Vitamin C is the highest level in all vitamins, Antioxidant capacity were determined higher than %90. Consequently; our work has confirmed that; *Angustifolia* fruit is the source of a powerful antioxidant. It has high radical scavenging. We believe that ıts frequent ingestion may reduce oxidative stress may be protective against the disease.

Key Words

Elaegeneus Angustofolia, antioxidant vitamin, antioxidant capacity, antiradical effect

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INTRODUCTION

Free radical; single unpaired electrons in atomic or molecular structure is the name given to parts. Very easy to exchange electrons with other molecules to such molecules capable "oxidizing molecules" or "reactive oxygen particles" is also called. The liberation or generation of reactive oxygen species (ROS) within the body cells may directly or indirectly contribute degenerative illness, such as the different cancer, cardiovascular neurological diseases [10], tumor formation [11], Parkinson [12]. Alzheimer [13], multiple sclerosis [14], and cataract [15]. Antioxidants stop the formation of free radicals in the human body, or adverse effects are those which do not. *Elaeagnus angustofolia* is thought of a plants with strong antioxidant content. *Elaeagnus angustifolia*, is in the family Elaeagnaceae. There for this study; aim that was determined to Antioxidant vitamin levels, anti-radical property against to MDA, which is an indicator of lipid peroxidation.

MATERIAL and METHODS

Reagent, Chemical And Instrumentation

Eleagnus angustifolia was provided to a plant supplier in Elazığ. All solvents were of analytical-grade reagents. They were purchased from Merck (Darmemsted, Germany), Sigma- Aldrich (st, Louis, Misosoria USA). As a reagent DPPH analysis for 2,2-diphenyl-1-picrylhydrazyl, methanol, ABTS disposal methods for 2,2'-azino-bis(3-ethyl benztiyoazol -6-sulfonic acid), $K_2S_2O_8$, OH radical disposal for H_2O_2, buthanol, EDTA, $FeCl_3$, ascorbic acid MDA for 0.05 M Tris-HCl pH 7.4 / 0.15 M KCl and 0.2% tween 20 with a buffer solution containing 1 mM hydrogen peroxide FR and 3 mM were used. All these spectral analysis was performed with a spectrophotometer Shimadzu UV model 1400. All the vitamin analysis was carry out with HPLC, liguid chromatographic system (Shimadazu) consisted of LC-10 AD^{VP} pumps, SIL-10AD^{VP}, degasser unit DGU-14A and Class VP software (Shimadzu, Kyota Japan), auto sampler, SIL-10AD^{V}column oven, a CTO-10AS^{VP} UV-visible, and detectorSPD-10A^{VP}. These apparatuses were connected via a communication module (model CBM-20A) and controlled by Shimadzu LC solution Workstation. As column Supelcosil LC 18 (15 × 4,6 mm, 5 mm, Sigma, USA) column was used. Vitamin A detection wavelength of 326 nm, 202 nm, vitamin E, D and K vitamins in the 265 nm were measured. For the vitamin A, D,E,K n-hegzan, methanol, acetonitrile were used. For the vitamin $HClO_4$ and pure water were used. It is wavelength 245 nm, colon $C_{18.}$

Extraction process

Extracted from plants was performed using methanol solvent. Extraction process of plant parts by weight of 2 grams in weight 10 times the solvents were made.

DPPH radical scavenging activity

The free radical scavenging effect of *Elaeagnus Angustofolia* extracts was assessed by the discoloration of a methanol solution of DPPH$^{\cdot}$ according to the method of [3]. Briefly; DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared at a concentration of 25 mg/L. 4 mL DPPH solution to the test tube was allowed before. With varying concentrations of the tube, then the solution containing the plant extract was transferred. 30 minutes at room temperature and were incubated in the dark. After incubation at 517 nm against blank consisting of methanol in absorbance were measured. As a control, was used a solution of 4 mL of DPPH. Decreasing absorbance of the remaining DPPH$^{\cdot}$ free radical scavenging activity of a solution that gives the amount. Not from the media from the following equation the amount of DPPH calculated:

$$\text{DPPH Percentage of Destruction\%}=[(A_0-A_1)/A_0] \times 100$$

A_0 was the absorbance of control, A_1 as he absorbance of the samples was taken.

Hydroxyl radical (OH$^{\cdot}$) destruction activity

Extract of the hydroxyl radical (OH•) destroying activities with some modifications according to the method[4] was performed. The reaction mixture 500 µL plant extract, 500 µL 3.6 mM deoxyribose, 200 µL 100 $FeCl_3$, 200 µL of 104 mM EDTA, 100 µL of 1 mM H_2O_2, and 100 µL of 1 mM ascorbic acid solution was created and vortex thoroughly stirred. 37 ºC in an oven for 1 hour in 1 ml of 2.8% on the held tubes and TCA was added to 1 mL of 1.0% TBA. Hot water bath 50ºC for 30 min according to the held samples butanol phase UV at 532 nm in absorbance were recorded. OH%

of Extermination Radical Activity = $[(A_0-A_1) / A_0]$ x 100, A_0 was the absorbance of control, A_1 as the was taken absorbance of the samples

ABTS destruction activity

This method was performed according to the indicated method [5]. Summarily; 2:45 mM $K_2S_2O_8$ and 7 mM ABTS (2,2'-azino-bis (3-ethylbenztiyoazol-6-sulfonic acid)) solutions at room temperature with stirring in a 1:1 ratio and incubated in the dark for 16 hours. This prepared solution of ABTS radical 734 nm by taking the absorbance at 1850±0.05 absorbance was diluted until an ethyl alcohol. This absorbance was used as the absorbance. Then 4 ml of this radical solution to the test tube was allowed. Onto this tube at room temperature by addition of various concentrations of the plant extracts were incubated in the dark for 2 hours. After this time the 734 nm absorbance of the samples in PBS (Phosphate buffer, pH = 7.4) were recorded against blank consisting of no descending from the medium absorbance represents the amount of the ABTS radicals. Extreme ABTS radicals in the environment that destroyed much of the calculated according to the following formula:

ABTS % of Extermination Activity = $[(A_0-A_1)/A_0]x100$

A_0 was the absorbance of control, A_1 as the absorbance of the samples was taken.

Lipid peroxidation (LPO) measurement of MDA-TBARS

MDA analysis of plant extracts. was made according to the method [6]. For this purpose: 0.05 M TRIS-HCl pH 7.4/0.15 M KCl and 0.2% Tween 20 with a buffer solution containing 1 mM hydrogen peroxide FR and 3 mL were prepared daily.

LPO for the measurement, 1 mL samples on after receipt of 0,6% TBA solution and 2 mL distilled water was added and vortexes. Then 90°C for 30 minutes and the reaction was allowed resulting pink color was extracted with 3 mL of n-butanol. Samples were centrifuged and the supernatant fraction obtained after centrifugation of the color density was measured in a spectrophotometer at 532 nm.

Vitamin (A,D,E,K) analysis

All the other parameters in this study were measured by high performance liquid chromatography (HPLC) using previously described methods for vitamin [7,8]. Briefly; Plant extract in 10 ml of 3/2 mixture of isopropanol in hexane to pieces obtained by centrifugation from the supernatants, 5 mL supernatant 25 mL cap tubes have been put into the mouth on a 5% KOH solution was added. After vortexing, 85 ºC at 15 min was allowed to stand. Tubes were removed and on cooling to room temperature, 5 ml of pure water was added and mixed. Unsaponifiable lipophilic molecules, was extracted with 10 mL of hexanes. The hexane phase was evaporated with a stream of nitrogen. 1 mL (50% + 50%, v/v) acetonitrile/methanol the mixture is dissolved auto sampler vial transferred then in a HPLC was injected.

Vitamin C analysis

Vitamin C analysis were measured by using previously described methods [9]. Briefly Plant extracts on samples taken. 0.2 ml 0,5 M $HClO_4$ was precipitated by the addition of proteins. This mixture was then vortexes. then by adding pure water on a total volume of 1 ml complete. After 15 minutes the mixture centrifuged (2500 rpm/min) and then 20 µL of the samples taken carefully from on supernatants were analyzed on HPLC.

Statistical analysis

Results are expressed as mean ± SD. Statistical analysis and comparison were performed by SPSS software (Ver 17.0). Analysis of variance (One sample T test) and an LSD (least significant difference) test were used to compare the MDA experimental groups with the controls. Level of statistical significance was set at $P<0.05$.

Findings

All date are presented in Table 4 and Figure 3.

DISCUSSION

Antioxidant flavonoids from bark of *Elaeagnus angustifolia* with in study; The antioxidant activities of the flavonoids were evaluated. Results suggested that compounds showed significant antioxidant potential [16].

Tablo 1. Anti-radical property %

Anti-radical property	100 µL	200 µL	400 µL	800 µL
ABTS radical destruction Activity %	92.01 ± 0.70	95.85 ± 0.53	96.21 ± 0.43	96.11 ± 0.33
Hydroxyl radical (OH•) Destruction Activity %	64.01 ± 0.53	70.93 ± 0.97	71.3 ± 1.47	71.64 ± 1.21
DPPH• radical scavenging activity %	93.35 ± 0.52	96.75 ± 0.52	96.98 ± **0.60**	96.75 ± 0.47

Tablo 2. Lipid peroxidation (LPO) analysis (nmol/ml)

Fenton Reagent N:3	**QuercetinN:3**	**Resveratrol N:3**	**Control N:3**	**Measurement N:3**
71.99 ± 1.12	15.12 ± 0.21	69.69 ± 0.27	14.95 ± 0.74	33.32 ± 0.52

According to our findings; DPPH radical scavenging activity and OH radical destruction, ABTS knockdown activity increased with concentration difference. This shows that high antioxidant feature of the plant. Date are presented in Table 1.

ROS-mediated oxidation of membrane lipids result in the formation of lipid peroxidation of membrane (LPO) product as MDA [14]. MDA (malondialdehyde) is generally considered to be degradation of polyunsaturated lipids [17]. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells [18].

The LPO analysis; measurements compared with in both the control group and the standard significant statistically difference was observed. $P<0.05$.

When the level of MDA-TBARS was compared with other groups as to the control group. It was observed that only in the group containing Fenton reagent that were the level of TBARS distinctively high. Date are presented in Table 2 and Figure 1.

The cellular antioxidant systems can be divided in two major groups; enzymatic and no enzymatic. Some no-enzymatic low-molecular-weight antioxidant compounds, such as ascorbic acid (AsA), a-tocopherol (vitamin E), and carotenoids are consumed and may fall below normal range.

Vitamin K1 was found at the lowest level. E vitamin with K_2 vitamin close to each other have been detected. The vitamin D2, vitamin E and K_2 vitamin approximately 4 times more, the vitamin K1 is approximately 8 times higher were found. Vitamin A level in fat soluble all vitamins the highest level of detected. Vitamin C is the highest

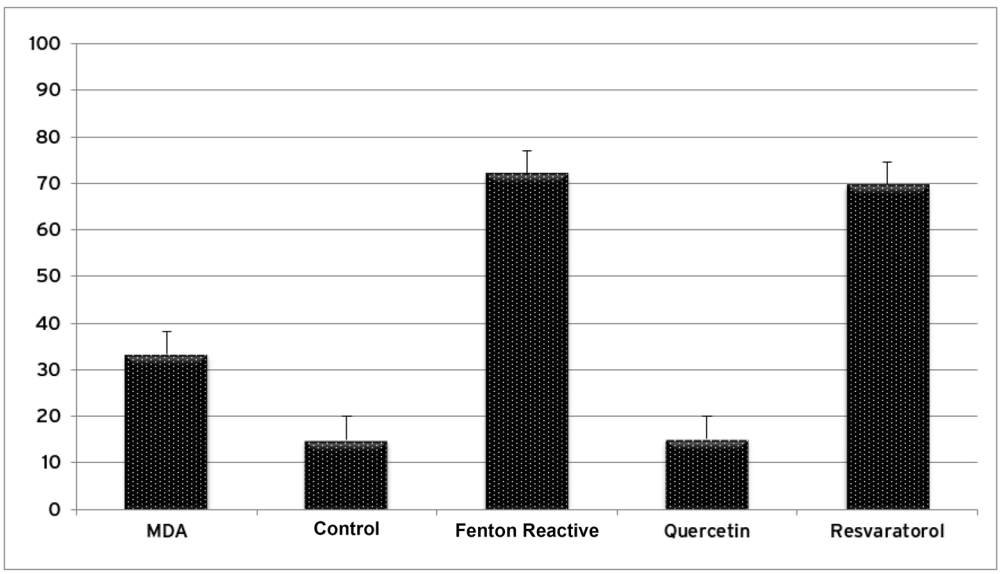

Figure 1. Inhibition activity of LPO formation *in vitro* environment of rutin, quercetin and resveratrol used in the level of 10 µM.

Tablo 3. Vitamin analysis.

Vitaminler N:3	Vitamin A (ATOK)	Vitamin E(RTOK)	Vitamin D	Vitamin K_2	Vitamin K_1
mg/ml	5.41 ± 0.21	0.11 ± 0.04	0,75 ± 0.17	0,1 ± 0.02	0.05

Tablo 4. Other parameters.

Vitamin D derivative component N:3	Ergosterol	Stigmasterol	Betastosterol
mg/ml	11 ± 0.14	4.2 ± 0.57	4.8 ± 0.40

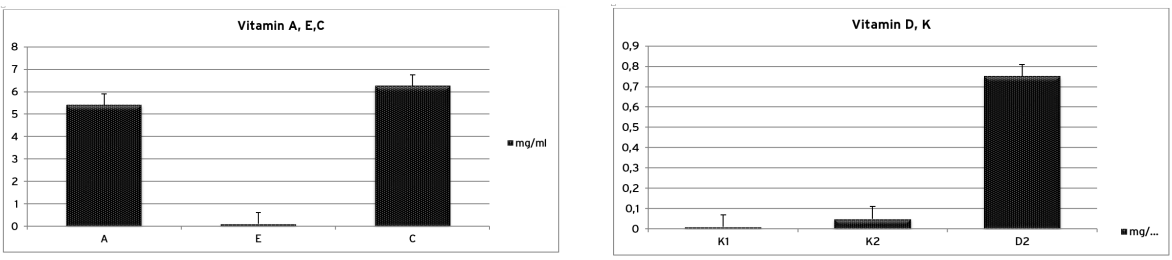

Figure 2. Vitamin used in the level of 20 μM.

level in all vitamins. Date are presented in table 3 and figure 2.1, 2.2

Vitamin D derivative compounds compared with each other; betastosterol and stigmasterol were at the same values. Ergosterol levels of other two components were detected at very low levels Date are presented in Table 4 and Figure 3

CONCLUSION

Since the synthetic antioxidants being used today possess adverse effects, it is of increasing importance to find out and derive natural antioxidants from herbal sources, to reveal their effects, and to use them. Our work and the information literatures confirmed that *Elaeagnus angustifolia* is a strong content. It has higher radical scavenging property. Frequent ingestion may reduce oxidative stress and it may be protective against the disease.

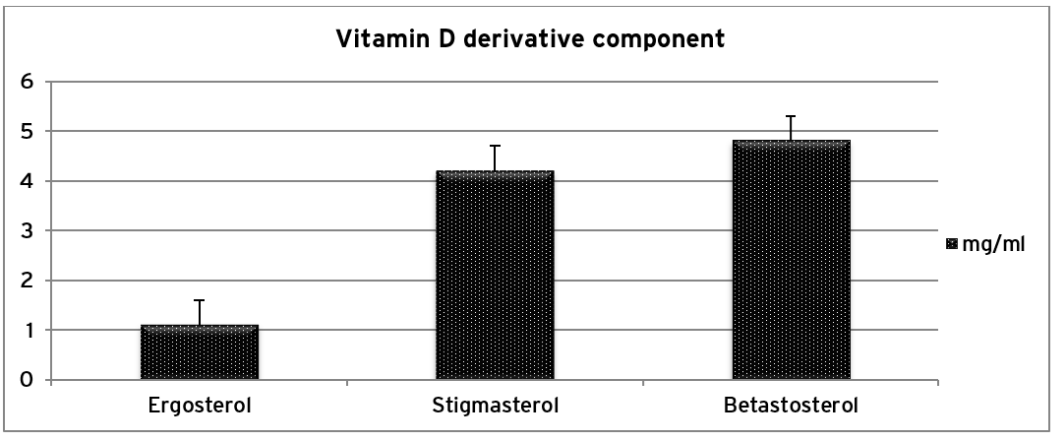

Figure 3. Vitamin used in the level of 20 μM.

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