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THE INVESTIGATION OF BIOCHEMICAL CONTENT OF *Elaeagnus angustifolia*

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ABSTRACT

Studies about herbal products are increasing every day due to their rich biochemical content. *Elaeagnus angustofolia* is one of the best known plant species to have a strong biochemical substance spectrum. This work was performed to identify the some biochemical content of Eleagnus angustofolia. In this study, vitamins A, E, and C, total sugar content, inverted sugar content, cellulose content, amount of total protein, and fatty acid properties were studied. Our investigations revealed that *Elaeagnus angustifolia* has a strong biochemical content.

Keywords: Eleagnus angustofolia, nutritional value.

Introduction

Elaeagnus angustifolia, which is in the family of Elaeagnaceae, is a usually thorny shrub or small tree that is able to grow up to 5–7 m in height. Its stems, buds, and leaves are densely covered by silvery to rusty scales. The plant has leaves that are alternate, lanceolate, having a length of 4–9 cm and width of 1-2.5 cm, along with a smooth margin. The flowers are of highly aromatic nature and grouped in clusters of 1-3, they possess a 1-cm long along with a four-lobed creamy yellow calyx; they begin to appear in the early periods of summer and are followed by a group fruit formation, the appearance being a small cherry-like drupe with a length of 1-1.7 cm, and orange-red formations are present, covered in silvery scales. The fruits are edible and have a sweet taste, though a dry, mealy texture is usually found [1]. This work was performed in order to determine the biochemical content of the species of *Eleagnus angustofolia*. In this study, we have performed the analyses of vitamins A, E, and C, total sugar content, inverted sugar content, cellulose content, total protein content, fatty acid content. Some relevant analyses were also performed and detailed in the experimental section.

Materials and methods

Reagents, chemicals, and instrumentation

Eleagnus angustifolia was obtained from a plant supplier in Elazığ district in Eastern Anatolia, Turkey. All solvents were of analytical-grade and were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, Missouri, USA). All the vitamin analyses were carried out with high-performance liquid chromatographic system (make of Shimadazu) consisting of LC–10 ADVP pumps, SIL-10ADVP, along with a degasser unit DGU-14A and Class VP software (Shimadzu, Kyoto Japan). The unit also has an autosampler, SIL-10ADVcolumn oven, a CTO-10ASVP UV-visible spectrophotometric detector SPD-10AVP. These instruments were connected via a communication module (model CBM-20A) and controlled by a Shimadzu LC solution workstation. As chromatographic column, a Supelcosil LC 18 (15 × 4,6 mm, 5 mm, Sigma, USA) was used. Vitamin A detection had a wavelength of 326 nm and 202 nm, whereas Vitamins E, D, and K were measured at 265 nm. For Vitamins A, D, E, and K, n-hexane, methanol, and acetonitrile were used as eluents in the chromatographic setting. For vitamin determinations, perchloric acid and distilled water were used. The wavelength to measure the absorption was 245 nm, and the chromatographic column used was a simply plain C_{18}. In order to perform the sugar analyses, methylene blue indicator, Fehling-1 reactant (copper(II) sulfate pentahydrate), Fehling-2 reactant (potassium sodium tartrate tetrahydrate) in sodium hydroxide, and phenolphthalein indicator dissolved in ethyl alcohol in 5% (w/w) were used; for cellulose analyses, potassium hydroxide, sulfuric acid, a desiccator to store the anhydrous material, and an incinerator were used. Total protein analyses were performed with the Folin's reagent. The fatty acid composition was elucidated with a Shimadzu gas chromatograph. Fatty acid methyl esters (FAMEs) were produced and introduced into the injection port of a Shimadzu GC 17 instrument (Kyoto, Japan). Machery-Nagel (Germany) capillary column of 25 m length, 0.25 µm inner diameter, and a Permo bond 25 µm thickness was used for this analysis. High-purity nitrogen was used as the carrier gas.

During analysis, mixtures of standard fatty acid methyl esters were injected and the retention time was determined for each fatty acid to learn the unique positions of standards included. After this process, mixtures of fatty acid methyl esters of the samples were analyzed. Examples of the solvents and reactants used include n-hexane, 5 mL of 2% $KHCO_3$, *etc*.

Vitamin A and E analyses

All the other parameters in this study were measured by high performance liquid chromatography (HPLC) using the methods previously described for these vitamins [2, 3]. Briefly describing, plant extracts produced from a mixture of 2:3 hexane – isopropanol (v/v) were divided into small portions and the supernatant was obtained by centrifuging the mixture. An aliquot of 5 mL was taken and put into capped tubes of 25 mL capacity. Then 5% KOH solution (w/w) was added. After vortexing the contents at 85 ºC for 15 min, the tube was allowed to stand. On cooling to room temperature, 5 mL of distilled water was added onto the tubes and then mixed. Unsaponifiable lipophilic molecules were extracted with 10 mL of a mixture of several hexanes. The hexane phase was evaporated under a stream of nitrogen. 1 mL (50%:50%, v/v) of acetonitrile / methanol mixture was used to dissolve the mixture and with the aid of the autosampler, the contents of the vial were introduced into the injection port of the HPLC instrument.

Vitamin C analysis

Vitamin C analysis was performed by using methods available in the literature [11]. 0.2 mL of plant extracts were taken. 0.5 M $HClO_4$ was used to precipitate the proteins. This mixture was vortexed and the total volume was completed up to 1 mL with distilled water. After 15 minutes, the mixture was centrifuged (2500 rpm / min) and then 20 µL of the supernatants was taken carefully to analyze with HPLC.

Total sugar, inverted sugar and sucrose analyses

Sugar analysis was performed according to the regulations outlined in TSE 1466 (Turkish standard), which is basically a volumetric method [12]. For inverted sugar measurements, an aliquot of 5 mL of extract solution was taken and was put into each flask, for a total of 4. The volume of the solution was completed to 50 mL by adding a sufficient amount of distilled water along with 5 drops of phenolphthalein solution. The mixture was neutralized with 0.1 N NaOH solution until the pink color of the solution persisted. The pH value was measured using a pH meter and the pH was adjusted to 7.0. A mixture of 5 mL of Fehling-1 solution and 5 mL of Fehling-2 solution was placed into a flask. 10 mL of distilled water was added. An aliquot of 5 mL of this solution was added into the previously prepared sample, which was being heated to boil. When boiling started, the flask was kept for 2 minutes. After 2 minutes, 10 drops of methylene blue solution were added. 5 mL of the sample solution was added to obtain a brick red color due to copper(I) oxide, which indicates the termination of the experiment. Inverted sugar content was calculated by using the formula below:

$$\text{Inverted sugar} = \frac{V_1 \times V_3 \times F \times 100}{V_4} \times \frac{V_2 \times g \text{ of sample}}{1000} \quad (1)$$

V_1 = volume of the solvent used to prepare the extract, $V_2 = V_{2.1} + V_{2.2}$, V_3 = the volume of solvent used to complete the final volume, F = factor of the solution used, V_4 = volume of the extract solution.

Total sugar analysis was performed by following the regulations of the Turkish Standards Institute with procedure number 1466 as the preferred volumetric analysis [12]. For inverted sugar, an aliquot of 5 mL from the extract solution (V_4) was taken and was added into each flask, for a total of 4. The total volume was made 50 mL by adding sufficient amount of distilled water. Another aliquot of 5 mL from this solution (V_4) was transferred into a conical flask and 2.5 mL of 2 M HCl was added. The flasks were placed in a container, into which a thermometer was submerged, and it was filled with hot water at 70 ºC, and the mixtures were kept for 5 minutes. Then they were cooled under tap water. A few drops of phenolphthalein were added, and the mixtured were neutralized with a solution of 0.1 N of NaOH. The pH value was measured by a pH meter to adjust to 7.0. Another 5.0 mL of sample from this solution received 5 mL of Fehling-1 solution and 5 mL Fehling-2 solution. Then 10 mL of distilled water was added. 5 mL of previously prepared sample ($V_{2.1}$) was added and the mixture was allowed to boil for two minutes after the initiation of boiling. Just through the end of this period, 10 drops of methylene blue solution were added. A portion of 5 mL of the sample was added to obtain brick red color owing to the formation of copper(I) oxide, and the experiment was terminated with the observation of the color. The calculation of total sugar content was performed by using the formula below:

$$\text{Total sugar} = \frac{V_1 \times V_3 \times F \times 100}{V_4} \times \frac{V_2 \times g \text{ of sample}}{1000} \quad (2)$$

V_1 = volume of the solvent used to prepare the extract, $V_2 = V_{2.1} + V_{2.2}$, V_3 = the volume of solvent used to complete the final volume, F = factor of the solution used, V_4 = volume of extract solution.

Sucrose levels were found by utilizing the following formula:

$$\text{Sucrose levels} = (\text{Total sugar} - \text{inverted sugar}) \times 0.95 \quad (3)$$

Determination of cellulose content

Cellulose analyses were measured according to TSE 4966 [6]. A certain amount of sample was taken, dried in an oven for 2 hours, and then crushed in a mortar. 3 grams (m) of the sample was taken and 60 mL of KOH was added. It was allowed to stand for half an hour under reflux. The samples were filtered into crucibles, which were treated with acetone for three times and two times with petroleum ether. The samples were oven-dried at 130 ^{0}C for 1 hour. A dessicator was employed for cooling and then the the crucibles were weighed (w_1). Then, another sample in the incinerator, which was kept at 500 ^{0}C for 1 hour, was cooled in a dessicator, and weighed (w_2).

The following formula was used to calculate the cellulose levels in the sample:

$$\text{Cellulose levels} = \frac{(w_1 - w_2)}{m} \quad (4)$$

Assessment of total protein amount

Protein amount was measured by spectrophotometric manner after Lowry et al [7]. 10 µL of the sample solution was added to the Lowry's reagent, and after waiting for 10 minutes, the sample was diluted with distilled water, and after the addition of Folin's reagent, the sample was measured at 760 nm.

Isolation of Fatty Acids and Gas Chromatographic Analyses of Fatty Acid Methyl Esters

Fatty acid composition was determined according to the method described in the literature [8]. Fatty acids were isolated by the addition of 10 mL of 3:2 (v / v) hexane / isopropanol mixture onto the liquid phase of the samples remaining after LPO measurement. Then the hexane phase was taken into separate test tubes and 5 mL 2% (w/w) methanolic sulfuric acid solution was added, followed by keeping at 55 °C for 12 hours. At the end of this time, 5 mL of 5% (w/w) sodium chloride solution was added and the fatty acid methyl esters were extracted with 5 mL of n-hexane. The mixture was treated with 5 mL of 2% $KHCO_3$ solution (w/w), then the n-hexane phase was evaporated under nitrogen stream [8], fatty acid methyl ester residues were dissolved in 1 mL of n-heptane and taken to autosampler vials. The analyses of fatty acid methyl esters were performed on a Shimadzu GC 17 instrument (Kyoto, Japan). A Machery-Nagel (Germany) capillary column of 25 m length, 0.25 µm inner diameter, and Permobond 25 µm thickness was used for this purpose. Nitrogen was used as the carrier gas. During the analysis, mixtures of standard fatty acid methyl esters were injected and the corresponding retention time was determined for each fatty acid. After this process, mixtures of fatty acid methyl esters of the samples were analyzed.

Statistical analysis

The results were expressed as mean± SD. Statistical analysis and relevant comparisons were performed by SPSS software (Version 17.0).

Findings

All data were presented in Table 4 and Figure 1.

Table 1. Vitamin analysis

Vitamin (N=3)	Vitamin C	Vitamin A (retinol)	Vitamin E (α-tocopherol)
$mg.mL^{-1}$	6.25 ± 0.14	10.1 ± 0.27	5.25 ± 0.24

Table 2. Total sugar, reducing sugar, sucrose, and cellulose levels in g per 100 g of total sugar in edible sources (N:3)

Sucrose	**Reducing sugar**	**Total sugar**	**Cellulose**
0.80 ± 0.81	29.77 ± 0.81	30.19 ± 0.58	4.5 ± 0.21

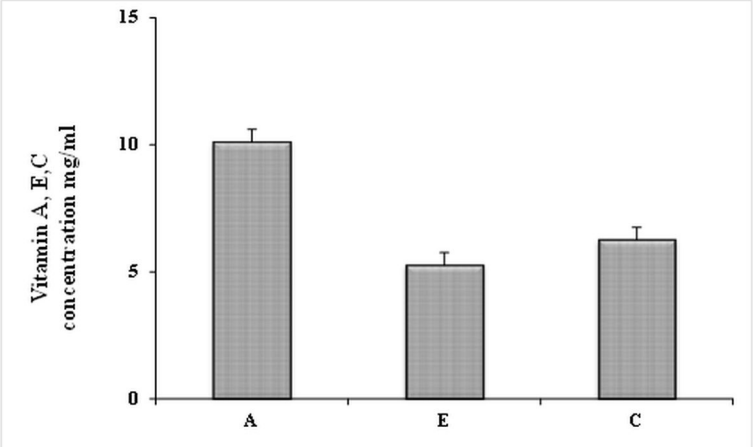

Figure 1. Vitamins used in the level of 20 µM.

Table 3. Total protein levels in mg/g protein of edible sources (N:3)

Total protein amount (N:3)	Value
$mg.g^{-1}$	0.66 ± 0.001

Table 4. Fatty acid composition (in percent) (N:1)

Detected fatty acid	Percentage
C16:0 palmitate	31.41
C16:1 ω-7 palmitoleate	10.40
C18:0 stearate	16.95
C18:1 ω-9 elaidate	8.64
C18:2;6,12, ω-6 linoleate	16.31
C18:3; 9,12.15, ω-3 linolenate	5.13
C20:4 ; 5, 8, 11, 14 ω-6 arachidonic acid	5.23

Discussion

In a previous communication, the authors have detected soluble sugars in *Elaeagnus angustifolia L* samples. The predominant sugars quantified were fructose at 27.1% and glucose at 22.3% of dry weight [9]. While we have found lower values of sucrose in the study, we have identified that the total sugar content was around 30%, which was clearly presented in Table 2. Vitamin A level was detected to be higher than that of vitamin E. Vitamin C was at the highest level among all vitamins present. The data were presented in Table 1 and Figure 1.

The chemical composition of lipophilic acid fractions from *E. angustifolia* cultivated in Russia were determined by GC-MS and HPLC. As a result, 13 species the fatty acid were determined in the extracts. These are namely lauric, tridecanoic, myristic, pentadecanoic, palmitic, palmitoleic, heptadecanoic, linoleic, linolenic, oleic, stearic, eicosanoic, and docosanoic acids [10]. While determining high levels of saturated fatty acids in our study; the unsaturated fatty acid content was observed to be at a lower level. We presented the data in Table 4.

Gonçarov *et al*. reported in their study about the determination of glycolipids and phospholipids of *Elaeagnus angustifiloia*; 9 glycolipids and 7 phospholipids were identified in seed fatty acid component. 8 glycolipids and 3 phospholipids have been identified as the fatty acid component in pericarp [11]. Also in their studies, they reported about the determination of some heavy metal content in *Elaeagnus angustifolia L.* According to their communication, Cu concentration was 25.39 µg / g, Fe concentration was 26.37 $\mu g.g^{-1}$, and Mn concentration was 11.70 $\mu g.mg^{-1}$. In this study, we have found a very low protein amount and the relevant data were presented in Table 3.

Conclusion

Our work and the literature information, in reference to the chemical and instrumental analyses, confirmed that *Elaeagnus angustifolia* has a strong content that might be useful in prospective studies which, we believe, can shed more light on this valuable herbaceous plant.

Declaration of interest

Contributing to our research, sugar and cellulose analyses were performed in Elazig Food Control laboratories for which the authors would like to express their thanks.

Conflict of Interest

We have no conflict of interest.

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