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Investigation of Antioxidant Activity and Phytochemical Compositions of *Celtis Tournefortii*

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ABSTRACT

Context: Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Because they have antioxidant property and protect our cells against oxidative damage. **Aim:** This work aimed to determination of phytochemical composition which total phenol content(TPC), total flavonoid content(TFC), total flavones content, alkaloid, acidity, pH, total carotenoid content, amount of carotene, β -carotene concentration, anthocyanin, amount of saponin and tannin, ascobic acid levels, mineral profile and antioxidant activity of fresh *Celtis tournefortii* due to the importance of secondary metabolites. **Material and method:** *Celtis tournefortii* was used for this study Phytochemical compositions were determined by spectrometric methods excluding phenolic acid and mineral profile. Antioxidant activity were determined by different antioxidant

method. Consequently: Celtis tournefortii may be an antioxidant source.

Key words: Antioxidant activity, *Celtis tournefortii*, Mineral contents, Phenolic acid, Phytochemical scavenging, Secondary metabolite.

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INTRODUCTION

Higher plants have both primary and secondary chemical metabolites. These metabolites very important evolution of plants.^{1,2} These metabolites play important role of biological activity. The most important of these biological activities is antioxidant property.

Celtis, commonly known as hackberries. *Celtis Tournefortii* is a genus from the Cannabaceae family. This kind is endemic to Eastern Europe: Ukraine, Croatia, Greece (including Crete, Sicily, Macedonia, Montenegro; Western Asia: Cyprus, northwestern Iran, northern Iraq, Turkey; and the Caucasus region: Azerbaijan. It can grow up to 6 meters in height and grows in plains and dry forests. *Celtis Tournefortii*, commonly known as the 'oriental hackberry' is a deciduous tree in the *Celtis* genus. This work was performed to determine the phytochemical composition, antioxidant activity and mineral profile of *Celtis Tournefortii*.

MATERIALS AND METHODS

Celtis Tournefortii, Elazig are obtained from the village Harput. After removing the core from the fresh fruit, analyses were performed. All solvents were of analytical-grade. They were purchased from Merck and Sigma-Aldrich. Spectral analyses were employed with a Jasco V-530 UV spectrophotometer. Tot phenolic, Tot flavonoid, Tot flavone, anthocyanin contents and antioxidant activity were determined with the obtained by methanol extract. We have used a filter paper from Schleicher & Schuell micro science589/3 run filter/circular ash less filter paper. Centrifuge was Hettich centrifuge universal 320. ICP-OES used for mineral analysis and phenol acids were determined Shimadzu mark HPLC.

Preparation of extraction

The core was removed from the sample and it was weighed 5 grams. It was placed in a beaker. 50 ml of methanol was added. The beaker was sealed with a plastic sheet and stirred for 24 hours. After 24 hours, it was filtering with filter paper. The supernatant separated. Onto residue was added 25 mL of methanol. The beaker was sealed with a plastic sheet and

was stirred for 24 hours by magnetic stirrer, then filtered. The second filtrate was combined with the first one.

Preparation of solutions

For total phenolic content, we used 1M Folin–Ciocalteu reagent, 5% Na_2CO_3 solution (w/v) was prepared.

For total flavonoid content, we prepared %5 NaNO₂ (sodium nitrite solution (w/v) and 10% AlCl₃ solution (w/v)

For Anthocyanin content, several buffer solutions were prepared. pH 1.0 buffer solution employed 125 mL 0.2 M KCl + 375 mL 0.2 M HCl, pH 4.5 buffer solution used 400 mL 1 M CH₃COONa + 240 mL 1 M HCl + 360 mL H₂O.

For determination of alkaloid amount, we used 10% acetic acid in ethanolic, ammonium hydroxide. For saponin amount determination, we used 20% aqueous ethanol, diethyl ether, n-butanol; for total carotenoid determination we used 95% ethanol, 5% KOH, diethyl ether; For carotene amounts we used ice-cooled acetone; β - carotene concentrations were determined with acetone, and anhydrous NaOH; For acidity measurement, we used 1% phenolphthalein, 0.1 N sodium hydroxide; For tannin amount, we used 0.1M ferric solution; for Vitamin C analysis we used 2,6-dichloro-phenolindophenol.

For the preparation of DPPH solution, 0,625g of DPPH (2,2-diphenyl-1-picrylhydrazyl) weight and diluting to 25 ml with ethanol. The resulting colored solution was further diluted with ethanol until it has an absorbance from 4.000 to 0.720 \pm 0.020 absorbance at 517 nm.

The ABTS^{*} solution was prepared by dissolving 6.6 mg of potassium peroxydisulfate and 30 mg of ABTS reagent in 7.8 mL in distilled water. The solution was incubated at room temperature 16 hours. The resulting colored solution was diluted with distilled water until it has an absorbance of 0.700 \pm 0.020 absorbance at 734 nm.

For determination of antioxidant capacity were prepared by reducing power of 0.2 M phosphate buffer (pH 6.6), 1% K_3 [Fe(CN)₆], 10 % C_2 HC- l_3O_2 and 0.1% (FeCl₃.6H₂O)

For determination of total antioxidant capacity by CUPRAC method; $CuCl_2$ solution was prepared at concentration of 10^{-5} M. $(NH_4)_2SO_4$ of buffer at pH 7.0 was prepared. Neocuproine (Nc) solution which is concentration of 7.5 x 10^{-3} M was prepared by dissolving 0.15 g of Nc in 99% ethanol and diluting to with water: ethanol (25:75 v:v).

Determination of total phenolic content

Antioxidant total phenolic content was assessed with the Folin-Ciocalteau assay.³ Briefly, 1mL of extract was mixed with 4.5 mL distilled water so, then 1 mL of 1 M Folin-Ciocalteaus reagent was added. The mixture was vortexed for 10 minute and was allowed to react for another 5 min period. Then, 2.5 mL of 7.5 % Na₂CO₃ solution was added. After incubation at room temperature for 2 hours, the absorbance of each mixture was measured at 760 nm. The same procedure was also used for the standard solution of Gallic acid (50, 100, 200, 400, 800 ppm) and a standard curve was obtained. In the results 1/10 diluted total phenolic contents were expressed as mg of Gallic acid equivalent/ g.

Phenolic acid profile

Phenolic and flavonoid analyses used in the methanolic extract (1:10) evaporated to dryness the residue remaining on the evaporator, and then 3 mL methanol was added. Then it was injected into HPLC. As using different phenolic standards, the retention times were determined. Using a calibration curve was determined the amount of phenolic acids in the sample. Results were identified as mg /g. The HPLC system operating conditions were as follows. Control Unit: Shimadzu CBM-20A, Pump Unit: Shimadzu LC-20AD X, Auto sampler: Shimadzu SIL-20A X, Degasser Shimadzu DGU-20A5, Column oven: Shimadzu CTO-10AS VP, Detector Shimadzu SPD-M20 to (DAD), Column: C18 5µm Clipeus 250x4.6mm, Temperature: 30°C, Injection volume was 20µL Mobile Phase A = acetic acid : water (3:97v/v), Mobile Phase B= water: acetonitrile: acetic acid(72:25:3v/v), Flow rate of 1 mL / min, Wavelength 280, 290, 355, 310, 329 nm.

Determination of total flavonoid content

Antioxidant total flavonoid content was determined with the aluminum chloride colorimetric assay³ 1ml of extract or standard solution of quercetin (50, 100, 200, 400, 800 ppm) was placed in a test tube, then 4 mL of distilled water was added, and the addition was finished with 0,3 ml %5 NaNO₂. After 5 minutes, 0.3 mL of 10% AlCl₃ was added. At the sixth minute, 2 mL of 1 M NaOH put in and the total volume was made up to 10 mL with distilled water. The solution was mixed. It was then incubated for 60 minutes at room temperature. Then the absorbance against the prepared reagent blank was determined at 510 nm with a UV-Vis spectrometer. The total flavonoid content of the extract was expressed as milligrams of Quercetin equivalents/g.

Determination of flavones content

The ingredient of flavones was determined by the modified colorimetric method as described by following method.⁴ Quercetin was used to standard calibration curve (50,100,200 ppm). 250 μ l of 2% aluminum chloride and 750 μ L of 5% sodium acetate were added to 0.1 mL the extract. The mixture is thoroughly homogenized and allowed to stand for 30 min at room temperature. The absorbance of all samples was measured at 440 nm using a Shimadzu UV-Vis spectrophotometer and the results are expressed in mg of Quercetin equivalent /g.

Determination of total anthocyanin content.

Total anthocyanin content was determined with by different pH.^{5.6} The tot anthocyanin content was calculated to be equivalent to cyanidin-3-glucoside.

; Total Anthocyanin content (mg /L)= A x MW x DF X 10^3 / ε x L

A = $(A_{510}nm - A_{700}nm) pH1.0 - (A_{510}nm - A_{700}nm) pH4.5$

MW is the molecular weight of anthocyanin (449.2), ε is cyanidin 3-glucoside's molar absorbance (26900), *L* is the cell path length (1 cm), DF is the dilution factor.

Determination of alkaloids

The determination of alkaloid amount was made according to described literature method by Harbone.⁷ In brief, 5 g sample was weighed and into a 250 mL beaker and 200 mL of 10% CH₃COOH in ethanol was added. The solution was covered and incubated at room temperature for 2 h. After then the solution was filtered and the filtrate was concentrated on a water bath to one quarter of the original volume. Concentrated NH₄OH was added drop wise to the extract and the precipitate was collected and washed with dilute and then filtered. The residues were dried and weighed.

Determination of saponins

The method employed for the determination of saponins was cited from following method.⁸ 8 gram sample weighted and was put into a flask and 100 mL of 20% aqueous ethanol solution was added. The sample was heated over a water bath at 55°C for 4 h with incessant stirring. The mixture was filtered and the residue was re-extracted with 100 ml of 20% ethanol. The total volume of combined extracts was reduced to 35 mL over water bath at 90°C. The concentrate was transferred into a separating funnel. Next, 20 mL of diethyl ether was added to the concentrate and shaken vigorously and then the ethereal layer was discarded. This process was repeated and then 60 mL of *n*-butanol was added to the extract. Finally, the solution was heated on a water bath and after evaporation, the samples were dried in the oven to a constant weight.

Determination of tannin content

5 gram samples were extracted with 20mL of warm water and filtered. Onto 0.1 mL of the filtrate was added to 0.1 ml of 0.1M ferric solution in an alkaline medium and allowed to stand for 30 minutes for color change, the absorbance was read at 760 nm and the amount of tannin was calculated from a standard calibration curve of Tannic acid. Results were expressed mg of Tannic acid equivalent/ g.⁹

Determination of total carotenoid content

0.5 g-sample was homogenized with 20 mL of 95% ethanol in an extraction tube. Then 2 mL of 5% KOH were added, and solution was filtered. The tube was stored in the dark for at 4 hours. 15 mL (C_2H_5)₂O was added and centrifuged. The ethereal supernatant was separated and its absorbance was read at 450 nm with the spectrophotometer.¹⁰ The total amount of carotenoid was calculated according to the following equation: Total carotenoid = $[(A_{max}/0.25)\times$ ethereal supernatant volume]/ sample weight.

Determination of total carotene content

Extraction of carotenoid components and chlorophylls was performed by a modified method.^{11,12} The method uses a sample of 0.5 g was mixed with 25 mL of ice-cooled acetone in the dark, the mixture was stored in the dark at -20 °C for 18 hours and the supernatant was filtered. Carotene, xanthophyll and chlorophylls a and b (µg/g) were determined spectrophotometrically at 470, 645 and 662 nm respectively by means of equations proposed by the owners of the method.¹³

Chlorophyll a = $11.75 {\rm A}_{_{662}}$ – $2.35 {\rm A}_{_{645}}$, Chlorophyll b = $18.61 {\rm A}_{_{645}}$ – $3.960 {\rm A}_{_{662}}$

Carotene = (1000A₄₇₀ – 2.270Ca – 81.4 Cb) /227 (C_a = chlorophyll a, C_b = chlorophyll b)

Determination of β - carotene amount

1.05 grams of a sample was weighed. We then added 0.2 g of anhydrous NaOH. After addition of 50 mL of acetone, the solution was mixed. After a while, another portion of 50 mL of acetone was added if the acetone solution looks colored, and solution was stirred. The color of the plant products was because of the extraction of the contents to acetone and the tube was kept at + 4 ° C for about 2 days in the refrigerator. To avoid exposure to light, the tube was wrapped with aluminum foil. After 2 days, the solution was taken from the refrigerator and was centrifuged. The reading of the samples at 475 nm in a spectrophotometer was used as a blank solution in acetone.^{14,15} The carotene concentration was calculated according to the following equation:

C (μ g/g)=A₄₇₄Vextractx1000/ ϵ xW A₄₇₄ sample absorbance V: extract volume passed to acetone, W: sample weight (g), ϵ : Molar absorption coefficient (2200 M /cmxL) in 1 cm cell.

Acidity, pH

For the determination of pH and free acidity, a 2g-sample were dissolved in 40 mL distilled water. The solutions were stirred magnetic stirrer at 30 min. and filtered. The filtered solutions were directly used for pH determination at room temperature using a pH meter. Furthermore, a 5 mL of the solution was taken in a flask and 2-3 drops of 1% phenolphthalein were added. Afterward, it was titrated with 0.1N sodium hydroxide for determining the acidity.

Analysis of ascorbic acid (vitamin c)

Ascorbic acid was determined by using methods available in the literature.¹⁶ The ascorbic content of fruits and vegetables can be estimated by reacting the sample with the stabilizing agent and mixing the decanted or filtered extract with an excess of 2.6-dichloro-phenolindophenol. The transmission is then read in 520 nm spectrophotometer. The sample amount was calculated and obtain the concentration of ascorbic acid was obtained from the standard curve. The result was stated as mg /100g solid or mg /100 mL liquid sample without decimal points.

Mineral profile

Mineral analysis was performed with ICP-OES. *Celtis Tournefortii* were dried for oven at 65°C 48 hours then milled. From the milled sample, weighed 0.5 grams, was taken into the microwave tube. Then 10 mL of HNO₃ (nitric acid) was added. After the effervescence had ceased, the tube was sealed, and the contents were solubilized in the microwave. After the burning process, the tube was opened, and the gas output was expected to end. Then, the solution was completed to 50 mL of ultra-pure water. Then it was introduced to the apparatus.

Radical scavenging power

Radical scavenging power of *Celtis tournefetti* were assessed by the method¹⁷ The reaction mixture had a total volume of 2.5 mL, which included 250 µL extract and 2250 µL of DPPH solution. The solutions were left in the dark at room temperature for 30 min and the resulting color was measured spectrophotometrically at 517 nm against blanks (250 µL ethanol + 2250 µL DPPH solution). A decreasing intensity of the color purple was related to a higher radical scavenging power percentage, which was calculated using the following equation; Radical scavenging power %=[Ao₃₀-As₃₀/Ao₃₀)*100 where, Ao₃₀ is absorbance of sample and As₃₀ is absorbance of blank at 30 min reaction time. Also, results were determined as using Trolox standard graphic and results were stated as mg of Trolox equivalent/ g fresh fruit

Reducing power method

Reducing power of samples was determined according to the method.¹⁸ Briefly; 1250 μ L of 0.2 M phosphate buffer (pH 6.6) and 1250 μ L of 1% K₃[Fe(CN)₆] were added to 250 μ L of sample solution and mixed gently. The mixtures were incubated at 50 °C in a water bath for 20 min. Reaction was stopped by adding 1250 μ L of 10% C₂HCl₃O₂ and the mixtures was transferred into tubes containing 1250 μ L distilled water and 250 μ L of 0.1% ferric chloride (FeCl₃.6H₂O). The resulting solutions were mixed well and, after 5 min, the color intensity read at 700 nm against blanks. Results, were given Trolox standard graphic (25, 50, 100, 250, 500 ppm concentration) were determined and results were stated as mg of Trolox equivalent/ g fresh fruit.

Assess of antioxidant capacity by ABTS* method

ABTS radical scavenging activity was determined by a method described literature of Re and cooperation.¹⁹ Onto 25 μ L extract solution was added 2475 μ L of ABTS* solution. The solutions were left in the dark at room temperature for 30 min and the resulting color was measured spectro-photometrically at 734 nm against blanks (25 μ L water + 2475 μ L ABTS solution). Results were determined as using Trolox standard graphic (25, 50, 100, 500 ppm concentration) and results were expressed as mg of Trolox equivalent/g fresh fruit.

Total Antioxidant capacity was determined by CUPRAC method

A series of Trolox solutions were prepared with different concentrations (25, 50, 100, 250 ppm). For determination of the antioxidant capacity, into a test tube were added 1 mL each of Cu(II), Nc, and $(NH_4)_2SO_4$ buffer solutions. Antioxidant sample (or standard) solution (*x* mL) and H₂O (1.1 - *x*) ml were added to the initial mixture so as to make the final volume 4.1 mL. The tubes were stoppered, and after half an hour, the absorbance at 450 nm (A_{450}) was recorded against a reagent blank. Results of antioxidant capacity was found from the Trolox calibration line concerned and the results were stated as mg Trolox equivalent/ g fresh fruit. The scheme for normal measurement is summarized as follows:1 mL of Cu(II) + 1 mL of Nc + 1 mL of buffer +*x* mL of antioxidant solution + (1.1 - *x*) mL of H₂O; total volume 4.1 mL, measure A_{450} against a reagent blank after half hour of reagent addition. (x =0.1 mL).²⁰

RESULTS AND DISCUSSION

All results presented in table 3 and graphic 1

Phytochemicals that showing antioxidant activity are polyphenols, flavonoids, sulfides, carotenoids [http://www.phytochemicals.info]. Polyphenols are natural chemical compounds in plants and they are excellent antioxidants. Because the electron reduction potential of their is lower than the electron reduction potential of oxygen radicals.^{21,22}

We determined total phenolic content of 6.674 ± 0.0012 mg of Gallic acid equivalent/g. Phenolic acids amount of were determined respectively as caffeic acid 0.756 mg/g, chlorogenic acid 6.882 mg / g, p-coumaric acid 1.968 mg/g, rutin 8.661 mg/g, ellagic acid 12.783mg /g, catechin 34.821 mg/g, myricetin 3.015 mg /g.

Flavonoids, groups of polyphenol compounds with known antioxidant properties have been isolated from plants.²³ In this work; we determined total flavonoid content to be 1.93267 ± 0.002 mg of quercetin equivalent/g methanolic extract.

According to a study published in 2012 year, from Celtis africana, were purification component namely trans-N-coumaroyltyramine (1), trans-N-feruloyltyramine (2), trans-N-caffeoyltyramine (3), lauric acid (4), oleic acid (5), palmitic acid (6), lupeol (7), β -cytosterol (8) and oleanolic acid (9), respectively.²⁴

Anthocyanins are water-soluble vacuolar pigments that may appear red, purple, or blue depending on the pH. They belong to a parent class of molecules called flavonoids synthesized, they are odorless and nearly flavorless, contributing to taste as a moderately astringent sensation. Anthocyanins occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits; anthocyanin's also act as powerful antioxidants. In this work we determined the total anthocyanin content 8.984 mg of Cyanidin 3-glucoside equivalent/ L methanolic extract.

Adedapo and co-workers determined that the phenolic, flavonoids, and proanthocyanidin contents of methanol extract of *Celtis africana* of leaves and stems. While total polyphenol content for leaves was 14±0.11 mg of Tannic acid/g, for stems it was 15.39±0.28 mg tannic acid/g. Total flavonoid content for leaves was 0.70 ± 0.02 mg quercetin/g of dry plant material while for stems to be 1.07 ± 0.007 mg of Quercetin/g of dry plant material. Total flavonol levels of leaves and stems the same value (0.1 mg of Quercetin/g of dry plant material. Proanthocyanidin content for leaves to be 0.37 ± 0.21 whereas for stems it was 4.58 ± 0.25 .²⁵

Reported that *Celtis timorensis* had a total phenol content 0.73%, also very high tannin contents (2.23%) were recorded in its leaf, besides, *Celtis timorensis* has higher ascorbic acid contents (14.71 mg /100g) in a study.²⁶

Saponins are a compounds structure containing a steroidal or triterpenoid aglycone and one or more sugar chains²⁷ and they exhibit antioxidant properties. In this work; saponin content were determined as 104.5123 mg/ g.

According to a work published in 2005 year, the investigators determined crude protein and saponin amounts of *Celtis africana* and *Celtis durandii*. The crude protein amount for *Celtis africana was* 28.19%, whereas for *Celtis durandii* it was 19.68%. Also saponin amount for *C. Africana* to be 12.33%, but for *C. durandii* to be 19.25 %.²⁸

Tannin is widely component in plants. They are phenolic compounds which high molecular weight. Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. They form complexes with proteins, carbohydrates, gelatin, and alkaloids. In this study; we determined that tannin amount 170.6153 mg of tannic acid equivalent/g.

Carotenoids are also powerful antioxidants that are beneficial to human health Previous studies have suggested that they can prevent or delay cancer and degenerative diseases in humans and animals by contributing to anti-oxidative defenses against metabolic oxidative by-products.^{29,30} In this work; we determined that total carotenoid content was 49.298 \pm 0.081 μg /g, total carotene amount was 140.31 \pm 0,119 μg /g and β - carotene amount was 10.2618 μg /g

In other studies, fatty acids, nutrients, organic acids, vitamin C and sugar content of Celtis tortufenetti lam were determined. Among organic fruit acids, citric acid was detected as the highest (63.59 g / kg) in the mesocarp, malic acid was determined as the highest (12.97 g/kg) in the endocarp. For the content of sugars, glucose was found to be higher (0.84-9.96 g /100g) than the other sugars. Palmitic acid content in the shell portion of the fruit 6.02%, palmitoleic acid content of 0.11%, the amount of stearic acid was found as 2.78%, the amount of oleic acid was foun to be 23.54%, and the amount of linoleic acid 64.93% linolenic acid content of 1.24, arachidic acid amount was 0.32 %. Celtis Tournefortii Lam. fruit, When the amount of tocopherol in different parts of alpha-tocopherol amount of bark 13.56 µg /g, the amount of gamma-tocopherol, 45.28 μ g/g and the amount of delta-tocopherol 1.27 μ g / g was determined.³¹ The antioxidant is a component which significantly delays or prevents oxidation the substrate. There are several sources of antioxidant: those that we can get from food and food supplements e.g. vitamin C, E, D and

β-carotene selenium and polyphenols. Plant extracts and their constitu-

Table 1: All analysis results

Total phenolic content	6.674±0.0012 mg of Gallic acid equivalent/gr
Total flavonoid content	$1.932\pm0.002~mg$ of Quercetin equivalent/gr
The flavonols content	1.00691 mg of Quercetin equivalent/ g
Total anthocyanin content	8.984 mg of Cyanidin 3-glucoside equivalent /L
Saponin amount	104.5123 mg/g
Tannin amount	170.6153 mg of tannic acid equivalent/g
Alkoloid amount	$3.125 \pm 0.007 \text{ g}$
Ascorbic acid (Vitamin C)	16.02±0.90 mg/100g
pН	5.98 ± 0.51
Acidity	0.5±0.1 %
Total carotenoid amount	$49.298 \pm 0.081 \ \mu g/g$
Chlorophyll a	4.0984 µg/g
Chlorophyll b	4.2565 μg / g
Carotene amount	140.31 ±0.119 µg/g
β - carotene amount	10.2618 μg/g

Table 2. Phenolic acid profile

Caffeic acid	0.756 mg/g
Chlorogenic acid	6.882 mg/g
p-coumaric	1.968 mg /g
Rutin	8.661mg/g
Ellagic acid	12.7863mg/g
Catechin	34.821 mg/g
Myricetin	3.015 mg/g

Table 3: Antioxidant activity results

Radical scavenging power	0.11± 0.0007 mg of Trolox equivalent/gr fresh fruit, 52.46 % inhibition
Reducing power	0.93±0.026 mg of Trolox equivalent /gr fresh fruit
Antioxidant capacity by ABTS* method	$\begin{array}{c} 3.3835 \pm 0.043 \text{ mg of Trolox equivalent/ gr} \\ \text{fresh fruit} \end{array}$
Total Antioxidant capacity by Cuprac method	7.0995 ± 0.0007 mg of Trolox equivalent / gr fresh fruit



Graphic 1. Mineral profile mg/100 g.

ents are natural source of antioxidants. They are located in different plant organs such as seeds, fruits, leaves and stems.³²

It was reported that *Celtis australis L. (CA), Celtis occidentalis L(CO)* had DPPH radical % inhibitions which was in *n*-butanol fraction of CA and 70.3%, *n*-butanol fraction of CO 65.9%, while in the ethanolic extract of CA was 67.2%, ethanolic extract of CO was 58.5%. The values in the aqueous extract CA and CO were 55.6% and 48.5% respectively.³³ In this study, methanolic extract of *Celtis Tournefortii* DPPH radical inhibition determined to be 52.46 %.

In a study determined that the antioxidant activity of methanolic exract from the leaves and stems of *Celtis africana*. In leaves extract antioxidant capacity by FRAB method was 695.98 \pm 33.47 units of µmol Fe (II) g⁻¹, in the stem extract antioxidant capacity by FRAB method was 407.60 \pm 36.86 units of µmol Fe (II) g⁻¹. At a concentration of 0.1 mg / mL the radical scavenging activity of methanolic extract of the leaves reached 64.95%, while at the same concentration, the value for the stem was 89.69%. The methanolic extracts of the leaves and stems of *C. africana* were fast and effective scavengers of the ABTS radical, the percentage inhibition was 98.8, 98.8, and 99.3% for the leaf extract, stem extract, and BHT respectively at 0.1 mg /mL concentration.²⁵

CONCLUSION

Phytochemicals are naturally present in many foods. *Celtis Tournefortii* has includes a lot of components. Their consumption may many benefits to health. *Celtis Tournefortii*, owing to the availably of vitamin C, phenolic, carotenoids, flavonoids and mineral like, potassium, nitrogen, magnesium, calcium, may be used as a dietary supplement.

Next we will study *in vivo* antioxidant activity. This will increase the importance of studying.

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CONFLICT OF INTEREST

We have no conflict of interest.

DECLARATION OF INTEREST

Contributing to research, thanks Apricot Research Institute, Malatya, Turkey.

ABBREVIATIONS USED

HPLC: High Performance Liquid Chromatography; g: gram; Na₂CO₃: Sodium Carbonate; NaNO₂: Sodium Nitrite; AlCl₃: Aluminum Chloride; KCl: Potassium Chloride; CH₃COONa: Sodium Acetate; HCl: Hydrochloric acid; H₂O: Water; KOH: Potassium hydroxide; K3[Fe(CN)6]: Potassium ferricyanide; C₂HCl₃O₂: Trichloroacetic acid; FeCl₃.6H2O: Ferric Chloride Hexahydrate; CuCl₂: Copper(II) chloride; ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometry.

REFERENCES

- Herbert RB. The biosynthesis of secondary metabolites. Chapman and Hall Ltd., London, New York/USA. 1981; pp. 178. https://doi.org/10.1007/978-94-009-5833-3.
- Duke JA. Handbook of phytochemical constituents of GRAS herbs and other economical plants. CRC Press, London, UK. 1992; pp. 302.
- Marinova D, Ribarova F. Atanassova M. Total phenolic and total flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical and Technology and Metallurgy (Sofia). 2005;40(3):255-60.
- Abdel-Hameed ESS. Total phenolic contents and free radical scavenging activity of certain Egyptian Ficus species leaf samples. Food Chemistry. 2009;114:1271-

7.https://doi.org/10.1016/j.foodchem.2008.11.005.

- Wrolstad R, Durst RW, Lee J. Tracking color and pigment changes in anthocyanin products. Food Science Technology. 2005;16:423-8. https://doi.org/10.1016/j. tifs.2005.03.019.
- Wrolstad RE, Decker E.A, Schwartz S.J, Sporns P. Hand book of Food Analytical Chemistry: Water, Proteins, Enzymes, Lipids and Carbohydrates, A John Wiley & Sons, Inc., NewJersey. 2005 Vol. 2.
- 7. Harborne, J.B. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London.1973;88-185.
- Obadoni B.O. Ochuko P.O. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global Journal Pure Applied Science. 2001;8:203-8.
- 9. Trease G.E Evans W.C. Pharmacogn., 13th.Braillière Tindall, London. 1989; pp. 290.
- Britton G. Workshop on carotenoid: The qualitative and quantitative analysis. Prince of Songkla University, Thailand. 2005; pp. 9-16.
- Yoshii Y, Hanyuda T, Wakana I, Miyaji K, Arai S, Ued K, Inouye I. "Carotenoid compositions of *Cladophora* balls (*Aegagropila linnaei*) and some members of the *Cladophorales* (*Ulvophyceae, chlorophyta*): Their taxonomic and evolutionary implication." Journal of Phycology. 2004;40:1170-7. https://doi.org/10.1111/ j.1529-8817.2004.03210.x.
- Dere S, Günes T, Sivaci R. "Spectrophotometric determination of chlorophyll-a, b and total carotenoid contents of some algae species using different solvents". Turkish Journal of Botany. 1998;22:13-7.
- Lichtenthaler H.K, Wellburn A.R. "Determination of total carotenoids and chlorophyll a and b of leaf extract in different solvents". Biochemical Society Transactions. 1983;11:591-2. https://doi.org/10.1042/bst0110591.
- Yanar M, Çelik M, Yanar Y, Kumlu M. Carotenoid Pigments Stabilization in the Fillet of Rainbow Trout (*Oncorhynchus mykiss*) During Frozen Storage. Turkish Journal of Biology. 1998;22(3):61-5.
- Metusalach J, Brown A, Shadidi F. Effect of stocking density on color charectistic and deposition of carotenoid in cultured *Arthic charr (Salvelinus alphinus)*. Food Chemistry. 1997;59(1):107-14. https://doi.org/10.1016/S0308-8146(96)00205-1.
- Ragnel J.C. Analytical Methods in Quality Control of Processed Fruit and Vegetables. United Nations Development programmer Food and Agriculture Organization of the United Nations. 1973.
- Brand-Williams W, Cuvelier M.E, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technology. 1995; pp. 25-30. https:// doi.org/10.1016/S0023-6438(95)80008-5.
- Oyaizu M. Antioxidative activities of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. Nippon Shokuhin Kogyo Gakkaish. 1988;35:771-5. https://doi.org/10.3136/nskkk1962.35.11_771.
- [19] Re R, Pellegrin N, Proteggente A, Pannala A, Yang M.C, Rice-Evan. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1999;26:1231-7. https://doi.org/10.1016/ S0891-5849(98)00315-3.
- [20] Apak R, Güçlü K, Özyürek M, Karademir S.E. 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. Journal of Agricultural Food Chemistry. 2004;52:7970-81. https://doi.org/10.1021/ jf048741x PMid:15612784.
- Grace S.C. Phenolic as antioxidants In Antioxidants and Reactive Oxygen Species in Plants. Blackwell Publishing, Oxford, UK. 2005; pp. 141-68. https://doi. org/10.1002/9780470988565.ch6.
- Bors W, Michel W, Saran M. Flavonoid antioxidants: rate constants for reactions with oxygen radicals. Methods Enzymology. 1994;234:420-9. https://doi. org/10.1016/0076-6879(94)34112-5.
- James O, Okafor P.N. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistria*. African Journal Biotechnology. 2008;7(3):129-3133.
- Al-Taweel A.M, Perveen S, El-Shafaea A.M, Fawzy G, Malik A, Afza N, Iqbal L, Latif M. Bioactive Phenolic Amides from *Celtis africana*. Molecules. 2012;17:2675-82. https://doi.org/10.3390/molecules17032675 PMid:22391601.
- Adedapo A.A, Jimoh F.O, Afoloyan A, Masika P. Antioxidant Properties of the Methanol Extracts of the Leaves and Stems of *Celtis africana*. Record Naural Product. 2009;3(1):23-31.
- Dasari R, Sathyavati D, Belide S.K, Reddy P, Abbulu K. Evaluation of antioxidant activity of two important memory enhancing medicinal plants *Celtis timorensis* and *Vanda spathulata*. Asian Journal of Pharmeceutical and Clinical Research. 2013;6(2):153-5.
- Kensi C.R. Saponins as vaccine adjuvants. Critic Review in Therapeutic Drug Carrier System. 1996;13(1-2):1-55.
- Burgess M.A, Chaipman C.A. Tree leaf chemical characters: selective pressures by folivorous primates and invertebrates. African Journal Ecology. 2005;43:242-50. https://doi.org/10.1111/j.1365-2028.2005.00578.x.
- Burtin, P. "Nutritional value of seaweeds". Electronic Journal of Environmental Agricultural Food Chemistry. 2003;2:498-503.
- 30. Tapiero H, Townsendand D.M, Tew K.D. "The role of carotenoids in the preven-

tion of human pathologies". Biomedicine & Pharmacotherapy. 2004;58:100-10. https://doi.org/10.1016/j.biopha.2003.12.006 PMid:14992791.

- Özrenk K, Gündoğdu M, Türkoğlu N, Gazioğlu-Şensoy R.I. Some Chemical Properties of Oriental Hackberry (*Celtis Tournefortii* Lam) Naturally Grown in Erzincan Region. Yuzuncu Yil University Journal of Agricultural Sciences. 2012;22(1):26-32.
- 32. Key A, Blazovic A, Rozlosnik N, Feher J, Petri G. Antioxidative Properties of

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Extracts From Smpervivum tectorum. Planta Medica. 1992;58:A661. https://doi. org/10.1055/s-2006-961674.

33. [33] El-Alf T, El-Gohary H.M.A, Hosny M, El-Mahdy D.A. A New Flavonoid C-Glycoside from *Celtis australis* L. and *Celtis occidentalis* L. Leaves and Potential Antioxidant and Cytotoxic Activities. Scientia Pharmaceutica. 2011;279(4):963-75. https://doi.org/10.3797/scipharm.1108-19 PMid:22145118 PMCid:PMC3221499.

SUMMARY

 Celtis tournefortii was used for this study Phytochemical compositions were determined by spectrometric methods excluding phenolic acid and mineral profile. Antioxidant activity were determined by different antioxidant method.