JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Phytochemical Evaluation of *Morus alba* Seeds and Cold Pressed Oil

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Abstract: This study has focused on *Morus alba,* which is grown in Turkey mostly for the production of fruit. *M. alba* seeds' analysis results showed that oil, protein, ash, ash insoluble in hydrochloric acid, and total carbohydrate were, 21.33±0.58 g, 21.58±0.13 g, 3.99±0.13 g, 0.9±0.00 g, 54.76±2.42 g for 100 g of sample respectively; the main minerals were calcium, phosphorus, and potassium. Dominating fatty acids were linoleic acid (80.56±0.22%), palmitic acid (7.96±0.06%), oleic acid (7.11±0.05%). The primary volatiles were l-limonene, 2,2-dimethyldecane, and hexanal. Sterol components were found as beta-sitosterol>delta-5-avenasterol>campesterol>cholestenol and the total sterols' amount was 5501.49±44.26 mg/kg. δ-tocopherol, γ-tocopherol, β-tocopherol, α-tocopherol, total phenolic content, the radical scavenging activity were 257.67±4.51 mg/kg, 18.23±0.11 mg/kg, 6.71±0.13 mg/kg, 3.23±0.06 mg/kg, 137.1±0.36 mgGAE/100 g oil, 19.9±0.46% respectively. Other properties were free fatty acid (1.55±0.89% oleic acid), peroxide value (3.23±0.55 meq O_2/kg of oil), p-anisidine value (1.18±0.55) and refractive index (40 °C) (1.4687±0.00). As a result, *M. alba* seeds and oil along with its fatty acid, tocopherol, sterol and mineral composition can be used as nutritional supplements.

Keywords : *Morus alba*, phytochemical properties, volatile oils, fatty acid composition, sterols and tocopherols

Submitted: October 13, 2018. **Accepted:** January 10, 2019.

Cite this: Ustun-Argon Z, İlhan N, Gökyer A, Büyükhelvacıgil-Öztürk S, Koparal B. Phytochemical Evaluation of Morus alba Seeds and Cold Pressed Oil. JOTCSA. 2019;6(1):41–50.

DOI: https://dx.doi.org/10.18596/jotcsa.470279.

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INTRODUCTION

The mulberry belongs to the flowering plants' genus Morus which is in the Moraceae family. The Morus genus has 24 species and at least one hundred known varieties belong to one subspecies. Mulberry plants can grow in various climate, soil and topographical conditions. Therefore, it can be found widely in the temperate, tropical or subtropical regions of both the Northern and the Southern hemisphere (1,2). Mulberry trees have been planted in Turkey for more than 400 years. According to the statistics in 2017, 74,383 tons of mulberry were cultivated from 2,713 trees in Turkey (3) and these trees represent three different species: *Morus alba (M. alba)* (%95), *Morus rubra* (3%) and *Morus nigra* (2%) (4). The white mulberry (*Morus alba*) is also found in China, Korea and later naturalized in Asia, Europe and America with a warm climates or subtropical zones. The Middle East, East and Southeast Asian countries are the main cultivation areas (4).

Mulberry fruits have different usages in traditional Turkish food products such as (mulberry fruit leather, mulberry churchkhela,

mulberry molasses), natural dyes and cosmetic products (1,5). The plant also has traditional medicinal purposes not only in Turkey but also in various countries of the world. Mulberry fruits can be used with anthelmintic, odontalgic, expectorant, laxative, emetic, hypoglycaemic effects and as a remedy for dysentery and oral lesions (6,7). In Chinese folk medicine, the fruits have been used for hypertension, arthritis, anemia, and diabetes treatment (2). Morus fruits also reported with their cooling and laxative effects, and applications for sore throat, fever, thirst, melancholia, and dyspepsia in Asian traditional therapies (8,9). Some important phenolic components which are found naturally in mulberries like anthocyanins, and other chemical components such as flavanoids, steroids, volatiles, trace elements, vitamins and amino acids, affect the physical and medical characteristics of the fruits such as color, neuro-protective effects, antioxidant, anti-inflammatory, and antimicrobial properties (2,10). The other parts of mulberries (leaves, bark, and branches) have been used in traditional medicine in various ways to protect the liver, strengthen joints, lower blood pressure, improve eyesight, tonify the blood, treat edema, gray hair and wheezing (10,11).

There have been some studies on some mulberry species' and some different parts of the plant like leaves, root, and bark (1). There are very limited studies which are especially focused on chemical properties of *M. alba* seeds and oil. This study aimed to show that native *M. alba* seeds and oil can be a new natural supplement alternative with its chemical properties. Therefore, in the present study, we have focused on some phytochemical and physicochemical properties of *M. alba*. The seeds were analysed for oil yield, trace elements, protein, total ash, and ash insoluble in hydrochloric acid. The seeds' cold pressed oil was analyzed for fatty acids and sterol compositions, volatile oils, antioxidant capacity, tocopherol compositions, and total phenols. The cold pressed oil was evaluated also for free fatty acid content, refractive index, peroxide, and p-anisidine value. The focus of this study to provide deeper scientific data for *M. alba* and evaluate the availability of native white mulberries for further medicinal and supplemental applications with their phytochemical properties.

MATERIAL and METHODS

Plant and oil samples

Commercially ripened mulberry samples were provided from Adıyaman, in the Tut Region in Turkey. Seeds were seperated from the fruits in place and sun-dried to represent the commercial raw material. Seed oil was extracted with the cold press method at Zade Vital Pharmaceuticals, Inc. The process temperature was under 40 °C. For the extraction no chemicals and heating applications were used and the whole process was completed according to GMP rules.

Chemicals

All the reagents were purchased from J.T. Baker and Sigma–Aldrich products which were of analytical or chromatographic grade. The water used for the analysis was provided by Millipore ultrapure water (Type I).

Determination of Protein, Total Ash, Ash Insoluble In Hydrochloric Acid, and Carbohydrate content

Total protein content was determined using the Kjeldahl's method and for crude protein calculation the nitrogen conversion factor was 6.25 (12). EP 8.0 method 2.4.16 was used for total ash analysis and EP 8.0 method 2.8.1 was used for ash insoluble in hydrochloric acid analysis. The total carbohydrate percentage was calculated by the difference of the total sample amount from protein, ash and ash insoluble in hydrochloric acid .

Determination of Trace Elements content

Trace elements analysis has been completed according to EPA 3051A, EN 14332 methods by using ICP-OES. For the analysis 7 mL HNO_3 65% and 1 mL H_2O_2 %30 were added onto 0.5 g of mulberry seed samples. The microwave digestion is conducted with the Milestone Ethos One oven and the microwave parameters were for steps 1 and 2; T1: 200 ˚C and T2: 110 ˚C Pressure: 45 bar with max power for 15 min. After the digestion process the samples were cooled down to the 25 °C and were diluted to 200 mL. Final analysis was done by using ICP-OES (Shimadzu ICPE-9000).

Fatty Acid Methyl Esters (FAME) Analysis and Sterol Composition

For determination of fatty acid methyl esters content (FAME) COI/T.20/Doc. No 33 for olive oils method was used (13). Retention time was used to identify the fatty acids, and for the quantitative analysis the area ratio under the relevant peak was used . The standard for the retention time was A 37 component mixture of FAME (Supelco). Shimadzu 2010 Plus GC-FID system , and a Restek Rt2560 capillary column (100 m x 0.25 mm ID x 0.2 µm) were used for the analysis. Split ratio was 1:100, injection temperature was 250°C and detector temperature was 260°C. The temperature was programmed as follows: At 140 °C, holding for 1 min and then increased to 240 °C at a rate of 4 °C/min and hold for 5 min.

Sterol analysis was completed with preparation of unsaponifiable matter and then isolation of the sterol fraction of the fatty oil according to EP

8.0 method 2.4.23. Sterol determination was done by using gas chromatography (Shimadzu 2010 Plus). The column was Teknokroma (TRB-Sterol) (30 m x 0.22 mm ID X 0.22 µm). Carrier gas was hydrogen, split ratio was 50:0 and flow rate was 40.0 cm/s. Column, injector, and detector (FID) temperatures were 260, 280 and 290 °C, respectively, and the injection volume was 1 µL for each analysis.

Free Fatty Acids, Peroxide Value, Refractive Index and p-Anisidine Value

Free fatty acid (FFA) content analysis was completed according to EP 8.0 method 2.5.1 and for peroxide value (PV) EP 8.0 method 2.5.5 was applied. Refractive index was determined by using EP 8.0 method 2.2.6 and Rudolph J57WR, for P-anisidine value A.O.C.S Official Method Cd 18-90 was used. The p-anisidine values were calculated using the following equation: p-A.V.=25*(1,2As-Ab)/m

Determination of Volatile Oils

Volatile compounds determined by Gas Chromatography-Mass Spectrometry (GC-MS). The equipment was a Shimadzu model GC – 2010 with mass selective detector QP 2010 plus – MS. The column was Restek Rxi-5ms, with 0.25 µm thickness, 30 m length, and 0.25 mm internal diameter. Volatile oils were identified with the database of the equipment. For analysis oven temperature was set to 40 °C, injection temperature to 250 °C in split mode with the split ratio 20, total flow was 40,8 mL/min, column flow was 1.80 mL/min. Oven temperature programmed as follow; holding the oven temperature at 40 °C for 3 min and increasing the temperature to 240 °C at a rate of 4 °C/min and holding for 5 min.

DPPH Free Radical-Scavenging Assay

The free radical scavenging activity was determined spectrophotometrically by the DPPH assay (14). Briefly, 1.95 mL of DPPH (0.025 mg/mL) which was prepared in methanol was added to 50 µL of methanolic solution of the sample. 30 min later, the absorbance was measured at 515 nm. As the control sample methanol (80%) was preferred. The following equation was used to calculate the capability of scavenging the DPPH radical :

$$DPPH\ radical\ scavenging\ effect\ (\%) = \frac{A\ control - A\ sample}{A\ control}\ x\ 100$$

$A_{Control}$: The initial concentration of the DPPH

A_{Sample} : The absorbance of the remaining concentration of DPPH in the presence of the extract and positive controls.

Determination of Total Phenolic Content (TPC)

Total phenolic content of the samples was determined using the method given in the study of Ferhat *et al.* (14,15). Gallic acid was used as the standard. For the analysis, 5 mL of distilled water and 1 mL of Folin–Ciocalteu reagent were added onto the 0.5 mL of methanolic extract solution in the test tube, and the tube was shaken. Four minutes later, 0.8 mL of Na_2CO_3 (7.5%) solution was added. The mixture was allowed to stand for 2 h and shaken occasionally. Absorbance of the sample was measured at 640 nm. The phenolic compounds concentrations were calculated according to the equation below which was obtained from the gallic acid standard graph.

Absorbance = 0.006 gallic acid (mg) – 0.021 (R^2=0.969)

Tocopherol Content

Tocopherols' stock solutions (1000 µg/mL) were prepared by using n-hexane as the solvent and the working standard solutions were diluted from the stock solution. The concentrations of the calibration solutions were between 1.25-200.00 µg/mL for all tocopherols. 20 µL injections were repeated for three times for each concentrations of tocopherols. Calibration graphs were completed using the data related with the peak areas. The linear regression equations were calculated according to the emission at 330 nm, the excitation at 290 nm wavelengths and corresponding concentrations. Approximately 0.500 g (±0,001 g) of seed oil sample was weighed into 10 mL volumetric flask and 8 mL n-hexane was added. The sample solution was kept in an ultrasonic bath for 5 minutes of sonication. The volume is completed to 10 mL by adding n-hexane and sonication was repeated for 5 minutes (15,16). For the HPLC analysis Lichrosorb Si 60, 250 x 4.0 mm column was used and the column temperature was 25±1 °C, flow rate was 0,8 mL/min, and mobile phase was hexane/2-propanol (99,5/0,5) (v/v). Tocopherol Set (Merck) was used as a standard which is including a four-vial pack containing α, β, γ, and δ-tocopherols.

RESULTS and DISCUSSION

Oil, Protein, Total Ash, Ash Insoluble In Hydrochloric Acid, Carbohydrate Content

The oil content of mulberry seeds was found to be 21%, protein was found to be 21,58±0,13 g, ash content was at 3,99±0,13 g, ash insoluble in hydrochloric acid level was at 0,9±0,00 g and total carbohydrate was at 54,76±2,42 g for 100 g seeds (Table 1). The total carbohydrate and the oil content determined in the seeds were with the highest amounts, respectively.

The results were compatible with the other results in the literature. For example, Absar *et al.* (17) mentioned that mulberry seeds contain 38 g of carbohydrate, 32 g of fat, and approximately 15 g of protein per 100 g of seed . Another study found the total carbohydrate at 47.58-43.17%, total lipid at 29.04-27.15%, total protein at 20.20-21.50%, ash at 6.05-5.10% for *M. alba* and *M. nigra,* respectively (8). In a study by Imran *et al.* (18), total carbohydrates were found with the highest range 13.83±1.20-17.96±1.54 g/100 g and it was followed by protein (0.96 ± 0.16-1.73 ± 0.10 g /100 g), lipid (0.48 ± 0.11-0.71 ± 0.07 g/100 g) and ash (0.46 ± 0.06-0.87 ± 0.12) for four different Morus species of Pakistan. *M. nigra* samples from Turkey showed a similar composition of nutrients with different percentages such as 42.4–46.6% carbohydrate, 27.5–33% crude oil, 20.2–22.5% crude protein and 3.5–6% ash (5). Total ash content gave an estimate of the total mineral content (19) also total ash and acid-insoluble ash contents are important indicators of the quality and the purity of herbal medicines. Total ash content is based on plant tissues and environmental sources such as soil and sand. Determination of total ash is not generally adequate to respresent the quality of the herbal supplements (20), therefore, acid-insoluble ash analysis has been conducted in this study. The contents of nutrients, as mentioned earlier depend on various effects such as variety, soil, geographical conditions, and absorbed minerals during growing season (5,8). Besides, most of the studies have been conducted with the whole fruits not solely with the seeds. The differences in the results can be explained with these factors. When our results are compared with the reported results of other studies it can be accepted that *M. alba* seeds can be a good source of these nutrients.

Trace elements

The main mineral components have essential activities in the body. Calcium and phosphorus are important especially for bones and tooth structure. Calcium has a role in activation of some enzymes and hormones and regulation of blood clotting (21). Phosphorus can be found in the structural composition of nucleic acids, ATP, GTP, nucleotide coenzymes, and is also important in maintenance of osmotic and acid–base balance, enzyme systems, and intermediary metabolism. Potassium has an important activity in excitability regulation of nerves and muscles and can be added to the diet to control the hypertension of people who use diuretics and can help to replace the excessive excretion of potassium (21). Minerals are also vital for physical and mental health and basic components of muscles, hemoglobin, soft tissues, nerves and blood cells (22). The differences in mineral composition of the fruits and the seeds can be explained with the variety of the cultivars and species. It also can be related with the environmental factors such as climate, soil, geographical conditions, fertilizers, cultural management techniques (2), harvesting, and storage conditions (23).

Mulberry seed trace element levels measured for 100 g seed as presented in Table 1 were: calcium (Ca) (821.00 ± 24.58 mg), phoshorus (P) (288.67 ± 7.51 mg), potassium (K) (288.50 ± 10.61 mg), iron (Fe) (11.97 ± 0.61 mg), magnesium (Mg) (243.33 ± 11.59 mg), and sodium (Na) (35.30 ± 0.46 mg). Ca was at the highest amount, and it was followed by P, K, and Mg, respectively. In a study by Liang *et al.* (2) with eight different mulberry cultivars from China (2) it has been found that K level was higher than other elements and it has been followed by P, Ca, Mg, and Fe with similar quantities in our study. Another study, which evaluated the mineral contents of different mulberry species, found the K amount between the range of 1731 ± 11.50 and 1270 ± 9.36 mg, while Ca was–576 ± 7.37-440 ± 3.21 mg , Na was 260 ± 3.86-280 ± 3.50 mg and Mg was 240 ± 2.90-360 ± 4.20 mg/100 g for *M. alba, M. nigra, M. laevigata* black and white fruits. Ercisli and Orhan (1) found the results for *M.alba, M. nigra, M. rubra* between the following ranges: P (226-247 mg/100 g), K (834-1668 mg/100 g), Ca (132-152 mg/100 g), Mg (116-115 mg/100 g), Na (59-61 mg/100 g). These studies also mentioned other elements such as Cu, Mn, Zn, Ni, Se, As, Cr being in lower quantities. The results showed that with its rich mineral composition mulberry seeds could be recognized as a valuable nutritional product.

Table 1. Nutrients profile of *M. alba* seeds (n=3)

Nutrient component	**Amount (g/100 g of seed)**
Total carbohydrate	54.76±2.42
Protein	21.58±0.13
Oil	21.33±0.58
Ash	3.99±0.13
Ash insoluble in hydrochloric acid	0.9±0.00
Trace elements	**Amounts (mg/100 g)**
Calcium	821.00±24.58
Phosphorus	288.67±7.51
Potassium	288.50±10.61
Magnesium	243.33±11.59

Sodium	35.30±0.46
Iron	11.97±0.61

Fatty Acid Composition

In our study, the main fatty acids of mulberry seed samples were found to be linoleic acid (80.56 ± 0.22%), palmitic acid (7.96 ± 0.06%), oleic acid (7.11 ± 0.05%) and stearic acid (3.45 ± 0.02%) and these four components comprised approximately 99% of the fatty acid composition. Fatty acid methyl ester composition can be seen in Table 2. PUFA was 81,07%, MUFA was 7.27%, USFA was 88.34%, and SFA was 11.64%.

In a study about *M. nigra* from Turkey, main fatty acids were found to be linoleic acid (73.74%), palmitic acid (10.25%), oleic acid (8.20%) and stearic acid (4.48%), similar to our study (5). Another study about *M. alba* from Bangladesh found the linoleic acid to be the most dominant fatty acid by 74.29%, and it has been followed by palmitic (10.60%) and stearic (5.61%) acids. This study did not mention oleic acid (24). According to the results of this study mulberry seeds oil contains one of the two essential oils which can not be produced in the human body. *M.alba*'s oil can be considered as a good source of linoleic acid since its deficiency can cause poor wound healing, hair loss, and dry hair (2) and the addition of omega-6 to the daily diet in 5-10% can reduce the cardiovascular disease risk (25).

It can be seen in different studies with different mulberry cultivars from different countries that linoleic, palmitic, oleic, and stearic acids are the most dominant fatty acids generally with different ranges in accordance with our study (1,2,8,26). The differences in the fatty acid compositions may be due to the differences in species and genetics. The environmental conditions are the other factors which affect fatty acid composition and these conditions can be more effective on fatty acid composition more than the plant varieties (2,5).

The other properties of the *M. alba* seeds oil are given at the Table 2. Free fatty acid (FFA) content was found to be 1.55 ± 0.89% oleic acid, peroxide value (PV) was 3.23 ± 0.55 meq O_2/kg oil, p-anisidine value 1.18 ± 0.55 and the refractive index (RI) (40 °C) was 1.4687 ± 0.00. FFA content was found lower than the other studies of Morus varieties in the literature which were between 2.15-2.34 and 2.87 ± 0.17. PV was also lower than the results of the same studies which found the PV as 1.65-2.05 and 6.33 ± 0.22 (5,24) and also was lower than the recommended value of commercially available edible vegetable oils (PV≤10) (27). There was not any study, to our knowledge, with the anisidine value of mulberry seed oil. Therefore, our results were compared with another study which evaluated the raspberry fruits. The values of our study were found to be lower than the other study which was conducted with rasperry samples which p-anisidine value was 14.3 (27). It can be explained with the lower level of the secondary oxidation products. RI was similar with the other studies at 29 °C 1.465-1.468 and 1.469 ± 0.002 at 50 °C (5,8). With the results of the quality parameters *M. alba* seeds oil can be considered safe to use as edible oil and as ingredient for supplements.

Sterol Composition

M. alba seed's oil sterol analysis results revealed beta-sitosterol most abundantly by 78.21±0.61 mg/kg. Other sterol components were delta-5-avenasterol (8.11 ± 0.42 mg/kg), campesterol (6.73 ± 0.42 mg/kg), cholestanol (3.50 ± 003 mg/kg), stigmasterol (1.09 ± 0.04 mg/kg), delta-7-avenasterol (0.90 ± 0.03 mg/kg), sitositanol (0.59 ± 0.06 mg/kg), clerosterol (0.55 ± 0.03 mg/kg) and total sterol was 5501.49 ± 44.26 mg/kg. The study about *M. nigra*'s sterol contents gave the results which indicated that the main sterol components were betasitosterol, Δ5-avenasterol, Δ5,23-stigmastadienol, clerosterol, sitostenol and Δ5,24-stigmastadienol. The rest of the sterol composition contained campesterol, stigmasterol, cholesterol and Δ7-stigmasterol in small amounts (5). Our results show similarity with the previous study. With the knowledge that consuming enriched foods with with phytosterols can help lowering the plasma cholesterol (21) and has many benefits for human health (5), with our results it can be said that mulberry seeds' oil can be considered as a source of phytosterols.

Tocopherol Content

Results of the analysis showed that *M. alba* seed oil contains four different types of tocopherols. These were δ-tocopherol, γ-tocopherol, β-tocopherol, α-tocopherol and their levels were 257.67 ± 4.51, 18.23 ± 0.11, 6.71 ± 0.13, 3.23 ± 0.06 mg/kg, respectively. Total tocopherol content was 285.84 ± 4.80 mg/kg. While δ-tocopherol was with the highest value, α-tocopherol showed the lowest value. Compared with other studies our results found higher than the parsley and cardamom cold pressed oils' tocopherol levels (28), also α-tocopherol level of Gecgel *et al.* (5)'s work which reported as 0.18 ± 0.01 mg/100 g value. Yılmaz and Durmaz's study (26) showed the same gradation among tocopherols with higher percentages. The optimal tocopherol contents in vegetable oils are needed for the stabilization and technological quality of these oils (26,29). From the nutritional perspective, tocopherols intake in daily diet is considered beneficial for different

treatments such as cardiovascular problems, cataract, and tocopherols show neuroprotective and anticancer properties with antioxidant activities (21).

Volatile Oils

In our study, we identified 34 different volatile components which are dominated with monoterpenes (54.46%), alkanes (16,91%) and aldehydes (10.97%). Alcohols (3.75%), acids (3.44%), esters (2.72%), ethers (2.58%), benzenes (2.54%), other components (1.68%), ketones (0.51%), miscellenous components (0.18%) also have been analyzed in *M. alba* volatile oil (Table 3). For the monoterpenes l-limonene, alpha pinene and 2-beta-pinene were found with the highest amount. Looking into the components generally l-limonene was found with the highest percentage and it has been followed by 2,2-dimethyl-decane and hexane. Among the other components propanoic acid, 1-hexanol, hexanal, 4-ethyl-1,2-dimethylbenzene, 2,2-dimethyl-1-propanol acetate and anethole percentage were more than 1%. The most dominant compound limonene is generally found in citrus fruits and it has been used in different treatments with its antibacterial, hepatoprotective, anticancer, gallstones dissolving and gastric acid neutralizing effects (30–32).

Table 2. Physicochemical properties of *M. alba.*

Fatty Acids (%)*		**Sterols (%)****	
Palmitic acid C 16:0	7.96±0.06	Beta-sitosterol	78.21±0.61
Palmitoleic acid C 16:1	0.07±0.01	Delta-5-avenasterol	8.11±0.42
Margaric acid C 17:0	0.07±0.00	Campesterol	6,73±0.42
Stearic acid C 18:0	3.45±0.02	Cholestanol	3.50±003
Oleic acid C 18:1	7.11±0.05	Stigmasterol	1.09±0.04
Linoleic acid C 18:2	80.56±0.22	Delta-7-avenasterol	0.90±0.03
Gamma linolenic acid C 18:3	0.01±0.02	Sitositanol	0.59±0.06
Alpha linolenic acid C 18:3	0.47±0.01	Clerosterol	0.55±0.03
Arashidic acid C 20:0	0.12±0.01	Total sterol (mg/kg)	5501.49±44.26
Eicosenoic acid C 20:1	0.08±0.01		
Eicosadienoic acid C 20:2	0.03±0.00	**Tocopherols (mg/kg)**	
Behenic acid C 22:0	0.04±0.00	δ-tocopherol	257.67±4.51
Erusic acid C 22:1	0.01±0.02	γ-tocopherol	18.23±0.11
Lignoseric acid C 24:0	0.01±0.02	β-tocopherol	6.71±0.13
		α-tocopherol	3.23±0.06
Free fatty acid (% oleic acid)	1.55±0.89	Total tocopherol	285,84±4.80
Peroxide value (meq O_2/kg oil))	3.23±0.55		
p-anisidine value	1.18±0.55		
Refractive index (40 °C)	1.4687±0.00.		

*Percentage of the fatty acids in the oil
** Percentage of the sterols in total sterols

Table 3. *M. alba* volatile oil components (n=3)

Acids	**(%)*****
Propanoic acid	2.61±0.08
Heptanoic acid	0.62±0.03
Isovaleric acid	0.21±0.01
Alcohols	
1-Hexanol	2.59±0.24
1-Octen-3-ol	0.51±0.04

Benzeneethanol	0.24±0.07
1-Pentanol	0.22±0.04
Benzenemethanol	0.18±0.01
Aldehydes	
Hexanal	9.15±0.26
Heptenal	0.94±0.05
Nonanal	0.32±0.02
Heptenal	0.30±0.09
Benzaldehyde	0.26±0.05
Alkanes	
2,2-dimethyldecane	15.25±0.36
Decane	0.68±0.10
2,2,3,3-tetramethylpentane	0.37±0.02
Dodecane	0.30±0.04
Pentylbenzene	0.17±0.03
3,3,4-trimethylhexane	0.12±0.02
Benzenes	
4-ethyl-1,2-dimethylbenzene	1.39±0.81
Styrene	0.98±0.13
1,2-dimethylbenzene	0.17±0.06
Esters	
2,2-dimethyl-1-propanol acetate	2.13±0.15
3-methyl-1-butanol acetate	0.60±0.04
Ethers	
Anethole	2.58±0.11
Ketones	
2-Heptanone	0.51±0.13
Monoterpenes	
l-limonene	49.50±0.88
Alpha pinene	2.63±0.09
2-beta-pinene	1.15±0.14
Alloocimene	0.34±0.02
Alpha thujene	0.32±0.03
Camphene	0.31±0.01
Delta-3-Carene	0.21±0.09
Miscellaneous	
Dihydro-2(3H)-Furanone	0.18±0.06

***The percentage of the specific oil among the total volatile oil content

Total Phenolic Content

In our study, total phenolic content was found to be 137.1 ± 0.36 mg GAE/100 g. Our results are competent with the study of Gecgel *et al.*(5) which analyzed the different mulberry seeds and found the total phenolic content to be in the range of 112.2–152.0 mg GAE/100 g. The results of Yilmaz and Durmaz's study (26) found the phenolic content for purple mulberry and black mulberry as 193 mg GAE and 178 mg GAE for 100 g seed oil respectively. The phenolic content in different studies changed between 100–150 mg GAE/100 g oil similar with our results. Our results were generally lower than the other studies as we used the seeds for analysis while the other studies were conducted with the whole fruits. Comparing the seeds and the whole fruit, mulberry fruits were found to be with one of the most powerful phenolic contents among ~~the~~ other fruits. Different mulberry cultivars showed very rich content of the total polyphenols such as *Morus atropurpurea* Roxb. (189.67-246.00 GAE mg/100 mg DW) (2), *M. alba* (1650±12.25 mg/100 g FW) (18), *M. nigra* (1422 mg GAE/100 g) and *M. rubra* (1035 mg GAE/100 g) (1). The differences between the

total phenolic contents depend on the varieties of the mulberries and the amount of the phenolics which passed to the oil during extraction (26). Also other factors can affect the level of the phenolic compound such as genotype, maturity at harvesting and environmental conditions (1,2,18).

Free Radical-Scavenging Activity

Free radicals are accepted to have an important role for health problems such as cardiovascular diseases and chronic pathologies also have a major effect on lipid peroxidation. The compounds with the antioxidant effects can be used to reduce the radicals (18). Edible oils are known with their antioxidant effects and also therefore it has been important to include these oils in the daily diet (26). In our study we evaluated the *M. alba* seeds' cold pressed oil for their antioxidant capacity with the DPPH radical which is mostly preferred for natural products. The antioxidant capacity was found 19.9±0.46%. In the studies which compared different mulberry cultivars, *M. alba* and mulberry pomace were found with the highest antioxidant capacity (18,26). Another study which focused on eight different mulberry cultivars from China found the free radical scavenging activity rates between 50-96%. In this study the whole fruits have been evaluated and it has been implicated that phenolic compounds such as anthocyanins, phenolic acids, and flavonoids are accepted as the major responsible component of the antioxidant activities in the fruits (2). The difference can be explained with the different parts of the fruits which were used for the analysis. Also different varieties of the fruits can effect the results (2).

CONCLUSION

The study analyzed M. alba seeds' ash levels and ash insoluble in hydrochloric acid, indicating that with the quality and purity of the material, *M. alba* seeds can be considered an appropriate raw material for herbal medicines. Trace elements of the seeds such as calcium, phosphorus, and potassium also can be accepted as a source of some minerals in the diet which are important for bones, dental structure and treatment of hypertension. Seeds contain approximately 21% of oil content with the dominance of linoleic acid (80.56%). Therefore cold pressed oil can be used in diets for supplementing this essential fatty acid. Volatile oils consist mostly of monoterpenes, alkanes and aldehydes and mulberry seed oil can be used with its l-limonene content for phytotherapeutical applications. Tocopherols and sterols, which have preventive effects for cardiovascular problems and antioxidant effects, were also found in remarkable amounts. *M. alba* seeds and its oil can be used in diets and nutritional supplements with its nutritional and phytotherapeutic effects. Since the studies about the mulberry seeds oil have been very limited, more researches with different cultivars are needed for further applications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by the Zade – Zade Vital İbn-i Sina R&D Center and DUAMER-Natural Products Research and Development Center. The authors wish to thank the Zade Vital Pharmaceutials for supplying the raw material and cold pressed oil.

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