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Chemical Composition and Antibacterial Activities of *Corylus avellana* L. Bioproducts Grown in Giresun-Türkiye

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Abstract

Hazelnut has become an important commercial product in recent years due to its worldwide applications in the pharmaceutical industry as well as in the confectionery and food industry. In addition, hazelnut shell is a waste material obtained after hazelnut harvest and mainly used as heating sources. However, its bioproducts are essentially a very important phytochemical source. In this study, the composition of phenolic compounds, carotenoids, tocopherols and fatty acids of different bioproducts of hazelnut plant were investigated. The highest phenolic compound content (2630.84 µg/g), and lutein amount (73.05 µg/g) were determined in green leafy cover. The major fatty acids were found to be as oleic acid (81.493%), linoleic acid (7.778%) and palmitic acid (6.408%), respectively. Total tocopherol concentration of hazelnut kernel was determined as 364.1 µg/g. The antimicrobial activities of hazelnut components were tested against eight different pathogenic bacteria. The compounds showed strong antimicrobial activity against both Gram (+) and Gram (-) bacteria which might be attributed to the rich phytochemical composition of hazelnut bioproducts. This work comprehensively summarized the chemical composition of hazelnut bioproducts and their antibacterial activity potential grown in Giresun-Türkiye region which had an important place in the global market.

Keywords: *Corylus avellana*, hazelnut, bioproducts, chemical composition, antimicrobial activity

1. Introduction

The hazelnut (*Corylus avellana* L.), which belongs to the Betulaceae family, is the most widely grown tree nut in the world after almonds [1, 2]. *C. avellana* L. is cultivated in Europe and neighboring areas of Asia, including Türkiye and the Caucasus Mountains. Providing approximately 70% of the total production, Türkiye is the world's largest hazelnut producer. It is followed by Italy (~16%), the United States (~4%), and Spain (~3%) [3-5]. According to the Ministry of Agriculture and Forestry of the Republic of Türkiye, 665 thousand tons of hazelnuts were produced in 2016, with 60.5% produced in Türkiye, and 1.1 million tons of hazelnuts were produced globally in 2020 [6].

About 400 *C. avellana* cultivars have been identified and 18 of them are cultivated in Türkiye. Among them, Tombul hazelnut, which is mostly cultivated in Giresun province, is the first class quality (Giresun quality); the other varieties are classified as second class quality (or Levant quality). For this reason, Tombul hazelnut is considered to be the most important commercial variety worldwide [3, 7].

Hazelnuts are a rich source of phytochemicals that exhibit high antioxidant and antibacterial activity. Phytochemicals are defined as non-nutritive but biologically active compounds found in plants. They generally consist of carotenoids, phenolic compounds, organosulfur compounds, nitrogen-containing compounds, and alkaloids [8, 9]. Phytochemicals are known to protect against the harmful effects of free radicals, as well as reduce the risk of developing certain types of cancer, coronary heart disease, stroke, osteoporosis, type 2 diabetes, inflammation, diabetes, insulin resistance, and sudden death [9]. It is known that most tree nuts, especially hazelnuts, contain some phenolics and carotenoids [8].

Thus, hazelnut consumption is associated with a reduced risk of cardiovascular disease events [10-12].

Hazelnut oil is another valuable component for the food industry. The oil content of the dry weight of Turkish hazelnuts differs according to the region and variety where it is cultivated, and it is around 60%. Hazelnut oil, oleic acid (73.6–82.6%), linoleic acid (9.8–16.6%), palmitic acid (4.1–6.8%), and stearic acid (1.6–6%), mainly consists of unsaturated fatty acids. For this reason, its oil has high oxidative stability and nutritional value [13-16].

Hazelnut oil has commercial importance due to its rich nutrients. It contains a significant amount of biologically active substances, such as α-tocopherol and β-sitosterol, in addition to beneficial unsaturated fatty acids. These natural components not only provide anti-oxidation and anti-aging properties to hazelnut oil but also improve immunity with consumption, prevent arteriosclerosis and regulate cholesterol [14, 15].

Alongside the considerable body of research focusing on hazelnut kernels and oil, there has been a notable surge in interest towards exploring hazelnut by-products, encompassing elements such as skin, hard shell, green leafy covering, and tree leaves. These bio-products are the outcomes yielded subsequent to a sequence of procedural stages encompassing roasting, crushing, shelling/peeling, and the culminating act of harvesting. Among these by-products, none of them has commercial value except the hazelnut hard shell, which is used as a heat source. Also, green leafy covers are removed from hazelnuts immediately after harvest and can be used as fertilizer for hazelnut trees [17]. As a result of previous studies, especially chemical content analysis showed that these by-products are considered sources of phytochemicals with biological activity [18, 19]. Although more than 5000 plant-derived phytochemicals have been identified, a large percentage is still unknown. To understand their health benefits, their phytochemicals need to be defined. By-products of tree nuts, which are cheaper sources than their kernels, contain rich phytochemicals with multifunctional properties such as antioxidant and free radical scavenging activities and, anticarcinogenic and antimutagenic effects. Because of this it is of great interest to include them in the diet as functional food and natural antioxidant [9, 20].

In the food industry, interest in the use of natural antimicrobial compounds is increasing day by day as consumers avoid chemical preservatives. In this context, it is important to determine the antimicrobial capacity of phenolic compounds [4]. There are studies on antiradical activities and antioxidant capacities in extracts of other hazelnut by-products such as Turkish hazelnut kernel, green leafy cover, and tree leaf [1, 4, 18, 21]. Many studies have been performed on the most commercially valuable hazelnut species. Among them, tombul hazelnut and its by-products, including its nutritional properties, chemical contents, biological activities, and agricultural cultivation play an important role.

The phenolic characterization of the kernel, kernel oil, hard shell, and green leafy cover of this commercially valuable hazelnut species, as well as the total carotenoid content, particularly β-carotene, lutein, and zeaxanthin content, of the cold-pressed oil, were determined in this study. Finally, the antibacterial activity of the methanol extract of hazelnut kernel, oil, and by-products (hard shell, green leafy cover) against Gram-positive and Gram-negative bacteria was evaluated. Although studies on hazelnut and its bio-products have been done before, one of the most important aspects of this research is that hazelnuts and bio-products collected in the same harvest period are subjected to chemical analysis to determine many components in the same study.

2. Materials and Methods

2.1. Plant materials

Hazelnut kernels, hazelnut hard shells, and hazelnut green leafy covers belonging to *C. avellana* species were obtained from Giresun center. Kernel oil is obtained by squeezing the kernel with cold press. Hazelnuts were crushed and the shells were ground by the knife mill for rapid size reduction (4000 rpm, for 2.0 minutes).

2.2. Chemicals / Reagents

All analytical grade phenolic standards used in the analysis were obtained from Sigma- Aldrich (St. Louis, MO, USA). All the reagents were purchased from Sigma–Aldrich and they were either chromatographic or analytical grade. FAME mix (Supelco FAME 37 Mix.lot: LC-07964) was used as a standard for the determination of the retention time of fatty acids.

For the determination of α-tocopherol, Merck standard was used. In addition, carotenoid standards (all-trans lutein and all-trans β-carotene), $CaCO_3$, pyrogallol, KOH, $CaCl_2$, and Na_2SO_4 were provided from Sigma Aldrich. All the solvents used in this study were Merck LC-grade (LiChrosolv® solvents).

2.3. LC–MS/MS analysis of phenolic compounds

The bioproducts of the nut (kernel, green leafy cover, and shell) were separated, cleaned, and dried in a 40°C, 48-hour hot-air oven. Methanol was utilized as the extraction solvent during the Soxhlet procedure. For Soxhlet extraction, 10 g samples (kernel, green leafy cover, and shell) of various nut bioproducts were ground in a blender and placed in a Soxhlet cartridge, 250 mL methanol was added to the device, and the system was activated. After this, the solvent mixture was filtered, and the solvent was then extracted using a rotary evaporator at 175 mbar and 40°C. The samples were finally diluted

in methanol for LC-MS/MS analysis. In LC-MS/MS analysis, the ODS Hypersil (4.6 250 mm, 5 m) column was utilized. Water with 0.1% formic acid and B methanol were employed as mobile phases. Using the previously known approach, analyses were conducted. Each sample was extracted three times and examined in conjunction with three parallel samples [22].

2.4. FAME analysis by GC-FID

The hazelnuts were cold pressed, no solvent was used to determine the oil composition and the temperature was kept below 40°C during this process as reported in the literature [23]. A cold press machine has been used with a capacity of 1kg/hour. Cold-pressed oil was stored in stainless steel tanks for one day and allowed to settle for sediments. Then, the oil was filtered using filter paper with 1 µm-filter paper. In the last step, cold-pressed oils were filled into 50 mL amber-coloured glass bottles and stored at 25°C until analysis.

The reference standard mixture was analyzed for the identification of fatty acids based on their retention times. Analysis was performed in duplicate, and the relative amount of each fatty acid was calculated over the total fatty acid content. FAME analysis was performed with an Agilent 7890 Series GC/FID. An Agilent J&W CP-Sil 88 column (100 m x 0.25 mm ID x 0.2 µm) and a 1:100 split ratio was used. The inlet and detector temperatures were 250°C and 260°C, respectively. The oven temperature was held at 140°C for 1 minute and then increased to 240°C at a rate of 4°C/min and held for 5 minutes.

2.5. Tocopherol Analysis

For the determination of tocopherol amounts hazelnut oil was obtained from hazelnut kernels by cold pressing method. The hazelnut oil sample was kept in the incubator at 35°C for 1 hour to ensure its viscous fluidity. Then, the oil sample was shaken vigorously for 30 seconds and diluted 1:3 with 2-propanol according to the previously developed method [24]. Finally, by vortexing for 30 seconds, 20 µL of this solution was injected into the chromatographic system for analysis. For HPLC analysis, an Agilent 1260 Series high-performance liquid chromatography instrument, analytical column (Zorbax Rx, 3 µ, 150 × 4.6 mm, Agilent Technologies) with a C_{18} guard column was utilized at a constant temperature of 40°C. Acetonitrile and methanol, (60:40, v:v) mobile phase mixture were used as an isocratic system [25].

2.6. Carotenoid analysis

A modified procedure was used to extract carotenoids from hazelnut kernel, shell, and green leafy cover samples based on previosuly used methods [26]. Briefly, 1.0 g of each sample was weighed, followed by the addition of 1.0 g of $CaCO_3$. The mixture was extracted in an ultrasonic bath (Elmasonic S80H) for 10 minutes at 30 °C using 20 mL of THF: DCM (1:1 v/v tetrahydrofuran: dichloromethane) containing 0.01% pyrogallol. The solution was then centrifuged at 5000 rpm for two minutes. The supernatant was saved, and the residue was re-extracted with new ethanol until the biomass lost its color. The mixed solutions were further filtered by vacuum filtration using 47 mm of 0.20 µm nylon filter paper (Sartorius). These were kept at -20°C as unsaponified extracts until the LC-MS/MS analysis.

The method was repeated on the identical samples, and then the filtered extracts were saponified with 10% methanolic KOH for two hours in the dark. To prevent the degradation of carotenoids, N_2 gas was used to flush the samples. To halt the saponification process, 10.0 mL of a 10% (w/v) Na_2SO_4 solution was added. Then, 10.0 mL of diethyl ether was added to the extracts to pool the carotenoid fraction. The collection of the top phase was performed three times. $CaCl_2$ was then added to the mixture to eliminate any remaining water. Next, the carotenoid extract was again filtered using nylon filter paper, and the solution was rotary-evaporated at 40 °C and 400 mbar (Stuart RE 400). Before LC-MS/MS analysis, the residue was dissolved in 5.0 mL of methanol and stored at -20ºC. Each experiment was conducted in triplicate.

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) fitted with an Atmospheric Pressure Chemical Ionization probe was used to evaluate carotenoids in this investigation (APCI). At a vaporization temperature of 350ºC, the mass spectrometer (Thermo Scientific/TSQ Quantum Access Max) was run in full scan mode from m/z 50 to 900. Carotenoids were separated using gradient elution utilizing a YMC, C_{30} column (4.6 x 250 mm, 5 µm). 70% methanol, 5% water (containing 0.1% formic acid), and 25% methyl-tert-butyl ether constituted the first mobile phase. It was adjusted to 60% methanol and 35% methyl-tert-butyl ether after 5 minutes. At the 10-minute mark, 45% methanol and 55% methyl-tert-butyl ether were used. 15 minutes later, methanol was reduced to 25% and Methyl-tert-butyl ether was dropped to 75%. The overall time for analysis was 15 minutes. Analytical standards were used to identify and determine the retention periods of lutein and β-carotene. Positive ion mode analysis was conducted and optimized using commercial lutein and β-carotene standards. SIM mode employed m/z values of 569, 551, and 459 for lutein and 537, 445, and 431 for β -carotene.

2.7. Antibacterial activity assay

In this study, four Gram-negative (*Enterobacter aerogenes* ATCC 3048, *Escherichia coli* ATCC 36218, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 13883 and four Gram-positive (*Bacillus cereus* ATCC 10876, *Bacillus megaterium*

ATCC14581, *Staphylococcus epidermis* ATCC 12228, *Methicillin-resistant Staphylococcus aureus* (MRSA) ATCC 6710 bacteria were used to test antibacterial activity of methanol extracts obtained from different parts of C. *avellana* L. The bacteria were supplied from the Culture Collection of Erzurum Technical University (Erzurum, Türkiye). Tryptic soy agar (TSA) and tryptic soy broth (TSB) supplied from Merck (Darmstadt, Germany) were used to culture bacteria.

The antibacterial activity was performed by the disc diffusion method. The studied samples and their concentrations were given as followed; hazelnut hard shell (22 mg/µL, 11 mg/µL, 7.3 mg/µL, 5.5 mg/µL), hazelnut kernels (33 mg/µL, 16.5 mg/µL, 11 mg/µL, 8.25 mg/µL), hazelnut green leafy covers (21 mg/µL, 10.5 mg/µL, 7 mg/µL, 5.25 mg/µL). As the concentration of the studies samples were prepared according to the obtained extracts from the extraction process, they exhibited some differences. Netilmicin (NET30) (30 µg/disc) and ofloxacin sulbactam (OFX) (10 µg/disc) were used as the positive control and methanol was used as the negative control. The cell suspension concentrations of tested bacteria were adjusted to a 0.5 McFarland standard. 100 µL of the bacterial culture was spread on TSA. Then sterilized paper discs (Whatman no.5, 6 mm dia) impregnated with 10 µL of each methanol extract at different concentrations were placed on the bacteria and incubated at 37 °C for 24 hours. The clear zones of the paper discs were measured and recorded. The microdilution method was used to determine the minimum inhibitory concentrations (MIC). Maxipime (Bristol-Myers Squibb) in concentrations between 500 and 7.81 µg/µL was used as a positive reference for microdilution assay [22]. The experiments were repeated three times.

3. Results and Discussion

The results of phenolic components and LC-MS/MS parameters are given in Table 1 and Table 2 respectively. Among the results we obtained, protocatechuic acid in the green leafy cover, vanillin and gallic acid in the hazelnut shell, protocatechuic acid, gallic acid, and ferulic acid in the hazelnut kernel is higher than the other phenolic compounds. It is noteworthy here that protocatechuic acid is very high in the bark.

The most detailed studies on the bioproducts of C. *avellana* L. species grown in Giresun were carried out by Alasalvar et al. [3, 8, 9, 18]. In one of these studies, Alasalvar et al. [18] investigated the free and esterified amounts of some phenolic acids (gallic, caffeic, p-coumaric, ferulic and sinapic acid) in the green leafy cover. For this purpose, two different types of extraction solvents, ethanol, and acetone were tried. In the extractions, free caffeic, ferulic and sinapic acids were not found in the samples. Gallic acid and p-coumaric acid amounts in ethanol extractions were found to be 253 and 38 µg/g extract, respectively. Although the results we found are slightly higher, the amounts are close to each other. However, according to our results, 18.71 µg/g caffeic acid and 137.36 µg/g ferulic acid per extract were found in the green leafy cover. Apart from these, a high amount of (2630.84 µg/g per extract) protocatechuic acid was detected in the green leafy cover. The amount of ellagic acid (191.80 µg/g extract) was also found to be relatively high compared to other phenolics.

Table 1. Phenolic compounds quantified in hazelnut (*Corylus avellena* L.) sample (µg/g).

Phenolic Compound	Green leafy cover	Shell	Kernel
Gallic Acid	419.46	913.20	44.94
Protocatechuic acid	2630.84	655.25	41.35
Protocatechuic aldehyde	8.03	238.54	8.05
Catechin	13.49	169.23	ND
Epicatechin	ND	ND	ND
Caffeic Acid	18.71	ND	ND
Vannilin	25.22	1364.69	16.22
Taxifolin	21.47	51.16	5.99
p-Cumaric Acid	41.47	95.39	14.16
Ferrulic Acid	137.36	ND	29.95
4-OH-Benzoic Acid	8.93	16.10	6.73
Salisilic Acid	8.17	17.37	7.34
Rosmarinic Acid	ND	ND	ND
Oleuropein	ND	ND	ND
Rutin	ND	3.13	2.09
Resveratrol	ND	0.41	ND
Ellagic Acid	191.80	ND	5.53
Syringic Acid	ND	ND	ND

ND: Not detected
*The values are mean of three replicate analyses and RSD values are < 3%.

In another study by Shahidi et al. [1], the amounts of the same phenolic acids (gallic, caffeic, p-coumaric, ferulic, and sinapic acid) in green leafy cover, kernel, and hazelnut shell were given as the sum of their free and esterified forms. In this study, total gallic, caffeic, p-coumaric, and ferulic acid amounts in hazelnut shells were found to be 3261, 212, 757, and 333 µg/g, respectively. In our study, the free gallic acid and p-coumaric acid amounts of the same phenolic acids were found to be 913.20 and 95.39 µg/g, respectively. Caffeic and ferulic acid could not be detected. In the same study, Shahidi et al. [1] analyzed the hazelnut kernel with its skin and found the total gallic, caffeic, p-coumaric, and ferulic acid amounts as 127, 81, 208, and 105 µg/g extract, respectively. In our results, caffeic acid, one of these phenolic compounds, could not be detected in free form. The amounts of free gallic, p-coumaric, and ferulic acid in the kernel were found to be 44.94, 14.16, and 29.95 µg/g extract, respectively.

When compared to the studies of Gültekin et al. [27] with hazelnut kernel, the amounts of gallic acid and protocatechuic acid found were close to each other. In our study, ferulic acid was found to be quite higher than the results they found, while the amount of rutin found by them seems to be much higher than we found. In the four hazelnut extracts they studied, gallic acid amounts were found to be 40.03 µg/g (min 24.83-max 65.92) on average, while the average of protocatechuic acid amounts was 57.07 µg/g (min 48.45-max 64.50). The average amount of ferulic acid is 2.47 µg/g (min 1.72-max 3.65), while the average amount of rutin is 11.4 µg/g (min 8.72-max 15.18). These amounts are in the same order as us; 44.94, 41.35, 29.95, and 2.09 µg/g. While p-coumaric acid was found to be 14.16 µg/g in our study, the average result of four hazelnuts in their study was 4.87 µg/g (min 2.23-max 7.50). While Gültekin et al. [27] detected catechin amounts between 12.00-20.08 µg/g and epicatechin between 1.53-5.81 µg/g, we could not detect catechin and epicatechin in our sample.

Table 2. LC-MS/MS parameters for phenolic compound standards

Phenolic compounds	R_t (min.)	MS [m/z]	MS/MS [m/z]	LOD (mg/L)	LOQ (mg/L)	Polarity
Gallic acid	8.92	169.7	80.50	0.061	0.203	-
			126.20			-
Protocatechuic acid	12.13	153.8	110.40	0.049	0.162	-
			92.50			-
Protocatechuic aldehyde	13.16	136.9	92.25	0.026	0.087	-
			108.20			-
Catechin	10.92	289.2	203.90	0.068	0.227	-
			245.70			-
Epicatechin	11.26	291.5	123.30	0.045	0.151	+
			139.30			+
Caffeic acid	15.27	179.7	135.20	0.047	0.157	-
			136.20			-
Vanilin	15.87	150.91	92.30	0.023	0.076	-
			136.10			-
Taxifolin	16.68	303.0	126.20	0.058	0.194	-
			285.50			-
p-coumaric acid	17.00	163.9	94.30	0.116	0.387	-
			120.20			-
Ferulic acid	17.19	193.35	134.10	0.061	0.204	-
			178.00			-
4-OH-benzoic acid	18.12	137.90	66.60	0.031	0.104	-
			94.60			-
Salicylic acid	18.13	137.14	65.51	0.030	0.099	-
			93.26			-
Rosmarinic acid	17.82	359.18	134.30	0.029	0.095	-
			162.20			-
Oleuropein	18.00	539.10	275.80	0.050	0.167	-
			377.50			-
Rutin	18.26	609.37	300.60	0.007	0.024	-
			301.70			-
Rezveratrol	18.45	228.98	107.20	0.030	0.099	+
			135.10			+
Ellagic acid	19.47	300.90	229.10	0.087	0.289	-
Syringic Acid	15.45	183.07	123.2	0.192	0.643	-
			77.3			

In another study by Pelvan et al. [28], gallic acid 1.09, proto-catechuic acid 0.07, salicylic acid 0.06, 4-Hydroxybenzoic acid 0.07, ferulic acid 0.03 µg/g were found in hazelnut kernels, while these values were found 2.20, 2.02, 0.36, 0.33 and 1.63 µg/g respectively in our samples.

In a study conducted with C. *avellana* in Poland, per µg/g kernel; gallic acid 4.1 µg/g and 11.1 µg/g, proto-catechuic acid 1.1 µg/g, catechin 0.5 µg/g and 1.0 µg/g, epicatechin 0.1 µg/g, while taxifolin and p-coumaric acid could not be detected [29]. The amounts of taxifolin and p-coumaric acid were determined as 0.29 µg/g and 0.69 µg/g samples, respectively. Ceylan et al. [30] also analyzed some free phenolics in hazelnut shells in the same study. Among the results they found, to be given as µg/g per sample; while gallic acid was 0.8 µg/g and 2.7 µg/g, catechin was 0.3 µg/g and 0.8 µg/g, proto-catechuic acid, epicatechin, taxifolin, and p-coumaric acid could not be determined. These phenolic compounds in our extracts in the same order as µg/g per sample; 20.13 µg/g, 3.73 µg/g, 14.45 µg/g, n.d., 1.13 µg/g, 2.10 µg/g. There are serious differences between these results.

In another study with hazelnut shells, gallic acid was 62.17 µg/g, proto-catechuric acid 22.13 µg/g, catechin 176.41 µg/g, epicatechin 17.11 µg/g per sample, while taxifolin and p-coumaric acid could not be detected. The amounts found for these phenolic compounds are many times higher than the amounts of catechin and epicatechin found in our study [31].

As can be seen from the comparison of the results, there are serious differences between the results given per µg/g extract or per µg/g sample. The reason for these differences may be due to the differences in the regions where the hazelnuts are collected, and the extract contents may change from year to year.

The predominant fatty acid was found to be oleic acid (81.49%) followed by linoleic acid (7.78%), palmitic acid (6.41%), and stearic acid (3.14%) as shown in Table 3. Tüfekçi and Karataş reported the fatty acid profiles (%) of hazelnut samples from different regions in the Black Sea [32]. In that study, it was apparent that the results did not significantly differ according to the regions. According to Taş and Gokmen [7], Giresun round hazelnuts had 80.1% oleic acid, 10.9% linoleic acid, 5.7% palmitic acid, and 2.4% stearic acid. In addition, Bacchetta rt al. [33] determined that the predominant fatty acid in European hazelnuts was oleic acid (80.63 percent), followed by linoleic acid (10.57 percent), palmitic acid (5.95 percent), and stearic acid (2.48 percent). Parcerisa et al. [34] reported that the fatty acid profiles of American hazelnut varieties were as follows: oleic acid ranged between 77.08 and 80.76 percent, linoleic acid ranged between 10.46 and 15.55 percent, palmitic acid ranged between 4.72 and 5.77 percent, and stearic acid ranged between 1.38 and 3.34 percent. These findings were moderately consistent with our own, with the variations perhaps attributable to harvesting season, growth circumstances, and geographical location.

Table 3. Fatty acid profiles (%) of hazelnut kernel (*Corylus avellana* L.) sample.

Fatty Acids	Area %
Lauric A.(C12:0)	0.00923
Myristic A. (C14:0)	0.0386
Pentadecanoic A.(C15:0)	0.00945
Palmitic A.(C16:0)	6.408
Palmitoleic A.(C16:1)	0.190
Heptadecanoic A. (C17:0)	0.0478
cis-10-heptadecanoic A. (C17:1)	0.0686
Stearic A. (C18:0)	3.141
Oleic A.(C18:1 cis)	81.493
Linolelaidic A.(C18:2 trans)	0.00912
Linoleic A.(C18:2 cis)	7.778
Arachidic A.(C20:0)	0.168
Linolenic A.(C18:3n3)	0.311
cis-11-eicosenoic A.(C20:1)	0.161
Heneicosanoic A.(C21:0)	0.0118
Behenic A.(C22:0)	0.0346
Arachidonic A.(C20:4)	0.0373
cis-5,8,11,14,17-eicosapentaenoic A.(C20:5)	0.0119
cis-4,7,10,13,16,19-docosahexaenoic A.(C22:6)	0.0128

*The values are mean of three replicate analyses and RSD values are < 5%.

In the literature, there are not many studies on carotenoids in hazelnut kernels, hazelnut shells, and green leafy cover. When the data on carotenoids in the hazelnut itself was examined, Alasalvar and Bolling [8] reported that carotenoids were not found in their studies on the fat-soluble parts of the hazelnut. In another study conducted by Kornsteiner et al. [35], in which they investigated tocopherol types and carotenoids in many dried nut cultivars, β-carotene and lutein were not detected in hazelnut. In the study of Durmaz and Gokmen [14], they found lutein (0.19 µg/g), and zeaxanthin (0.88 µg/g) in hazelnut oil. In the studies of Stuetz et al. [36] on raw and fried nuts, the total lutein-zeaxanthin was found to be 1.69 µg/g in raw hazelnuts, while β-carotene was found to be 0.26 µg/g.

Table 4. Effect of saponification on the amount of carotenoids in hazelnut (*C. avellena* L.) samples.

Hazelnut samples	**Green Leafy Cover**		**Shell**		**Kernel**	
Carotenoids	**Before saponification**	**After saponification**	**Before saponification**	**After saponification**	**Before saponification**	**After saponification**
*Lutein (µg/g)	73.05	13.77	ND	2.57	ND	2.67
β-carotene (µg/g)	ND	ND	ND	ND	ND	ND

ND: Not detected
*The values are mean of three replicate analyses and RSD values are < 2%.

As can be seen, although hazelnut is an oily food source, it is not rich in carotenoids. There are mostly tocopherol types in this oil. Apart from these, no study has been found on carotenoids in green leafy cover or hazelnut shells. In our study, carotenoids could not be detected in the kernel and shell. However, when saponification was applied, 2.67 µg/g lutein was detected in the hazelnut kernel and 2.57 µg/g in the shell. The amounts found after saponification is higher than the values in the literature, but this shows that lutein is found in hazelnut in ester form, not in free form. On the other hand, 73.05 µg/g of lutein was determined without saponification, and 13.77 µg/g after saponification. The results were summarized in Table 4. The amount of lutein found in the free form of green leafy cover is very high compared to the amounts found in studies with hazelnuts in the literature. This means that hundreds of tons of chocolate chips left over from the hazelnut traded every year can be evaluated in terms of lutein.

The results we found in our study; were 284.8±22.8 µg/g for α-tocopherol, 2.1±0.4 µg/g for γ-tocopherol, 77.2 µg/g for β+Δ- tocopherols and 364.1 as total tocopherol. It is seen that α-Tocopherol values are within normal limits. However, the total amount of tocopherol, excluding α-tocopherol, seems to be higher than the amount of tocopherol found in other studies. This may be due to the fact that α-tocopherol is more resistant to heat than other tocopherols and the cold pressing method prevents the degradation of heat-sensitive tocopherols. The results for tocopherols are given in Table 5. Since it is stated that the amount of tocopherol in the oils obtained by the cold press method is approximately 20% higher than the amounts obtained by the soxhlet extraction, the cold pressing method was used in order to determine the tocopherols in the study [37]. Among the 17 varieties of C. *avellana*, it was determined in the studies that the variety with the highest amount of tocopherol was Tombul [38]. However, it was also stated that the amount of tocopherol in hazelnuts showed significant differences from year to year.

Table 5. Tocopherol profiles of hazelnut kernel (*C. avellena* L.) sample (µg/g).

Sample	α-Tocopherol	γ-Tocopherol	B+Δ- Tocopherol	Total Tocopherol
Tombul Hazelnut	284.8	2.1	77.2	364.1

*The values are mean of three replicate analyses and RSD values are < 5%.

In one of the studies, α- tocopherol and β + γ- Tocopherol amounts of Tombul hazelnut were found as 248.3 and 33.5 µg/g respectively, while the total amount of tocopherol was given as 281.7 µg/g [5, 7]. In another study, the amounts found for α-Tocopherol, γ-Tocopherol, δ-Tocopherol, and total tocopherol were given as 38.4, 0.61, 3.08, and 41.4 µg/g, respectively [38].

Taş and Gökmen [7] found the amounts of α-tocopherol, β + γ- tocopherol, and total tocopherols for Tombul hazelnut harvested in 2013 as 36.3, 4.90 and 41.2 µg/g respectively. When the same study was repeated for hazelnuts harvested in 2014, these values were found to be 16.0, 6.03, and 22.0 µg/g respectively. The study of Parcerisa [34] stated that the amount of α-Tocopherol was found as 303.8 for Tombul hazelnut grown in Türkiye.

Although there are changes in tocopherol amounts according to the harvest years, it is seen that the amount of a-tocopherol varies between 160-384 µg/g, and total tocopherol amounts between 282-414 µg/g in the Tombul hazelnut variety collected from Türkiye.

3.1. Antibacterial activity of hazelnut bioproducts

According to the results of antibacterial results, the methanol extracts of all the hazelnut bioproducts (hazelnut hard shell, hazelnut kernels, hazelnut green leafy covers) had moderate antibacterial activity against Gram-negative and Gram-positive bacteria. The MIC values of hazelnut hard shell extract against all the bacteria were determined as 7.3 mg/µL. The MIC values of hazelnut kernels were determined as 11 mg/µL against all the bacteria except *P. aeruginosa* and *K. pneumoniae*. The concentration of 16.5 mg/µL of hazel nutmeat extract inhibited *P. aeruginosa* and *K. pneumoniae*. Hazelnut green leafy covers extract showed higher inhibition rates at lower concentrations compared to other extracts. The MIC values were 7 mg/µL for *E. aeuriginosa*, *P. aeruginosa*, *S. epidermis*, and MRSA; 5.25 mg/µL for *E. coli*, *K. pneumoniae*, and *B. cereus*; 2.625 mg/µL for *B. megaterium*. The results of antibacterial experiments were given in Table 6.

Nowadays, there has been growing interest in natural antimicrobial compounds because of discovering the adverse health effects of synthetic food preservatives. *Corylus colurna* and its wastes such as hazelnut hard shell and hazelnut green leafy covers have been evaluated as an antimicrobial agent in previously performed studies [4, 30, 39- 41]. Oliveira et al. [4] reported the aqueous leaf extracts of different hazel (C. *avellana* L.) cultivars (Cv. M. Bollwiller, Fertille de Coutard, and Daviana) exhibited significant antimicrobial activity at concentrations between 0.1 mg/µL and 100 mg/µL *against B. cereus, B. subtilis, S. aureus, P. aeruginosa, E. coli, K. pneumoniae, C. albicans*, and *C. Neoformans*.

In another study performed by Oliveira et al. [40], the aqueous extracts of hazelnut kernels were reported that high antimicrobial activity was only found against Gram-positive bacteria (*B. cereus*, *B. subtilis*, and *S. aureus*, MIC values of 0.1 mg/µL) and Gram-negative bacteria and fungi were found to be resistant to the extracts at all the assayed concentrations. Kirbaslar et al. [39] reported that Turkish nuts and seeds showed strong antimicrobial activity against the Gram-positive and Gram-negative bacteria and the fungi, however, they did not indicate the concentrations of the tested material. In research performed by Ceylan et al. [30], *C. colurna* extracts prepared with petroleum ether, dichloromethane, methanol, and water reported that all the extracts at the concentration of 5 mg/µL had antimicrobial activity against different bacteria. Özaslan et al. [41] reported that green leafy cover (279 mg/µL) exhibited an antibacterial effect *against E. faecalis, K. pneumoniae L. monocytogenes, S. epidermidis, S. aureus*, and *B. subtilis* and green leaves of nutz (320 mg/µL) against *K. pneumoniae* and *S. aureus*. The results obtained from this study were compatible with the literature in terms of showing antibacterial activity. However, all studies in the literature were different from each other in terms of the degrees of antibacterial activity and the concentrations, at which the studied samples exhibited activity [19]. The antibacterial activity of *C. colurna* and its derivatives can be explained by the bioactive compounds, they contain such as phenolic compounds [42, 43], cyclic diarylheptanoids, quinic acid, flavonoid, and citric acid [17, 44], carpinontriol [45] etc. The differences between the results of different research can be explained by geographical and climatical differences [19].

Table 6. The antibacterial activity of *C. avellana* L. bioproducts.

Bacteria		**Hazelnut hard shell**			**Hazelnut kernels**			**Hazelnut green leafy covers**				**Positive controls**	
		11 mg/µL	7.3 mg/µL	5.5 mg/µL	16.5 mg/µL	11 mg/µL	8.25 mg/µL	10.5 mg/µL	7 mg/µL	5.25 mg/µL	2.625 mg/µL	NET30	OFX
Gram -	*E. aerogenes*	8	**7**	-	9	**7**	-	10	**8**	-	-	20	20
	E. coli	9	**7**	-	10	**8**	-	13	10	**7**	-	19	20
	P. aeruginosa	8	**8**	-	**8**	-	-	11	**8**	-	-	19	19
	K. pneumoniae	10	**7**	-	**9**	-	-	15	12	**8**	-	20	19
Gram +	*B. cereus*	11	**8**	-	11	**9**	-	12	9	**7**	-	20	21
	B. megaterium	11	**9**	-	10	**8**	-	14	11	8	**7**	21	21
	S. epidermis	10	**8**	-	10	**7**	-	11	**8**	-	-	18	20
	MRSA	9	**7**	-	9	**7**	-	11	**7**	-	-	18	19

Netilmicin (NET30) (30 µg/disc), * Ofloxacin sulbactam (OFX) (10 µg/disc)

Minimum inhibitory concentration (MIC) values (mm) obtained from disc dilution values were indicated in bold in the table.

4. Conclusion

This study tried to investigate all components of the hazelnut, including the shell, which was considered a waste product. This study provides significant information for food processors and the industry to evaluate hazelnut bioproducts for their needs. In addition, the nutritional and technological quality of hazelnut bioproducts from nations with low production rates is comparable or even superior, making them competitive on the worldwide market. Besides, the antibacterial activity of all components of hazelnut makes them more preferable for use as a food additive. In this connection, the study provides a comprehensive investigation of the Giresun-grown hazelnut bioproducts.

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Author's Contributions

Mehmet Emin Şeker.: Designed the study, worked on phenolic, tocopherol and carotenoid extractions.
Ayşegül Erdoğan: Performed GC-FID analyzes of oil analyzes and carotenoid extractions.
Emriye Ay: Carried out phenolic extractions.
Derya Efe: Carried out antimicrobial analysis.
Rena Hüseyinoğlu: Involved in identification and collection of the *C. Avellana*. All authors contributed to the writing of the article, read and approved the final version of the article.

Ethics

There are no ethical issues after the publication of this manuscript.

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