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ORIGINAL ARTICLE

Analysis of Anticancer Taxanes in Turkish Hazelnut (*Corylus avellana* L.) Genotypes Using High-Performance Liquid Chromatography

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ABSTRACT

Objectives: This study aimed to investigate the anticancer taxane profiles of edible and non-edible parts of seven Turkish hazelnut (*Corylus avellana* L.) genotypes. Hazelnut is one of the healthy foods rich in nutrients and antioxidants. Its regular consumption is associated with a reduced risk of coronary heart disease and cancer. Hazelnut has been described as a plant source that produces taxanes which are widely used in many cancers. Türkiye is a homeland of hazelnut culture and has its own cultivars. Investigation of anticancer taxane profiles in different parts of Turkish hazelnut genotypes is important to show the potential and value of this plant from the perspective of the pharmaceutical and food industries.

Materials and Methods: In this study, green leafy covers (GLCs) and hard shells (HSs) (non-edible parts), skinless kernels (SKs), brown-skins (BSs), and brown-skinned kernels (BSKs) (edible parts) of Çakıldak, Sivri, Tombul, Palaz, and Kalınkara as standard and Ham and Sivri Yağlı as local genotypes were used. The five parts of each genotype were ground to powder and eliminated to a size of less than 80 mesh. Each part was extracted using hexane and methanol for 10-deacetylbaccatin III (10-DAB III), baccatin III (BAC III), cephalomannine, and paclitaxel analyses in three replicates. Samples and standards were analyzed by acetonitrile: water gradient method on NOVA Spher 100 Phenyl-Hexyl C18 column inhigh-performance liquid chromatography reverse phase system with 228 nm ultraviolet detector and 1.0 mL/min flow rate. Microsoft Office Excel, 2016, and analysis of variance Jamovi Version 2.3 were used for statistical and data analysis, consecutively.

Results: Hazelnut parts differed to a very high degree from each other in terms of the highest amount of 10- DAB III (Ham HSs, 9,15 µg/g), BAC III (Kalınkara BSs, 7.24 µg/g), cephalomannine (Sivri Yağlı BSs, 6.37 µg/g), and paclitaxel (Ham BSKs, 4.36 µg/g) they contained. While HSs, BSKs, and BSs were rich in taxanes in all of the analyzed genotypes, SKs, and GLCs remain limited for anticancer taxanes.

Conclusion: This is the first report that revealed the differences in taxane contents of Turkish hazelnuts including previously untested standard and local genotypes and their parts. Significant differences between genotype and hazelnut parts are expected to highlight the health benefits of consuming raw Turkish hazelnut with BSs and their possible use as a functional food. These results add more information to elucidate the bioactive potential of Turkish hazelnuts and their by-products and provide a promising resource for the food and pharmaceutical industry with an anticancer perspective.

Keywords: *Corylus avellana*, taxanes, anticancer, functional foods, by-products

INTRODUCTION

Among nut species, Turkish hazelnut (*Corylus avellana* L.), is the most valuable tree nut crop worldwide.[1] Hazelnut species exhibit distribution mainly in Türkiye, Italy, Spain, Portugal, France, and some parts of the USA.[2] For the cultivation of high-quality hazelnut varieties, Türkiye has suitable growing conditions.[3] With 80% of cultivated hazelnut area in the world, Turkey is the leading producer and alone meets about 69% of global hazelnut production (776 thousand tonnes).[4]

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Hazelnut fruit is divided into three parts; these are green leafy cover (GLC), hard shell (HS), and hazelnut kernel which can be consumed raw [with brown-skin (BS)] or roasted (without BS). In addition to their role as a popular snack both in Türkiye and worldwide, approximately 90% of hazelnuts are utilized as an ingredient in a variety of processed foods, especially in confectionery and bakery products.[5] The kernel, a main product of hazelnuts, plays a special role in human nutrition and health due to its particular composition.[6-8] The distinctive nutritional and sensory characteristics of hazelnuts render them an incomparable and optimal ingredient for food products. However, the kernel constitutes less than 50% of the total nut weight, and substantial by-products (GLC, HS, and BS) are removed during harvesting and processing.[1,9] Among these by-products, none has nearly significant commercial value. The HS, a majority of the by-products, is mainly used as a low-value heating source, and the GLCs are rarely used as organic fertilizers.[3,5,10] The use of by-products as readily available in diverse bioactive compounds and functional food ingredients could become a valuable source.[9,10] Therefore, in recent years, the number of studies that focused on investigating the utilization of hazelnut by-products has increased.[10-12] Studies on HSs and BSs mainly investigate the extraction, characterization, and identification of substantial bioactive molecules such as phenolic compounds, dietary fiber, cellulosic compounds, activated carbon, and pigments.[5,13-15] In addition, researchers found that GLC extracts contained bioactive compounds that have high antioxidant, antibacterial, and free radical scavenger properties.[3,16] It has been shown that hazelnut is also a source of anti-cancer taxanes.[17-21]

Paclitaxel, also known as Taxol® is a representative of a class of diterpenes taxanes, which are widely used as a chemotherapeutic agent to stabilize microtubules in many types of cancer. Since the discovery of its antitumoral activity, Taxol® has been approved as a chemotherapeutic drug for the treatment of ovarian, breast, lung, bladder, prostate, melanoma, esophageal, and other types of solid tumors.[22] It has also been used to treat acquired immunodeficiency syndrome-related Kaposi sarcoma.[23] Taxol® is originally extracted in low yield from the bark of *Taxus brevifolia*. Chemical and partial synthesis of Taxol® from its precursors, 10-deacetylbaccatin III (10-DAB III), baccatin III (BAC III), and cephalomannine are also very expensive and time-consuming.[19] Although it was generally considered a particular metabolite of *Taxus* spp., paclitaxel extraction was carried out from hazelnuts.[17] For more than two decades hazelnut and its by-products have been studied from the aspect of anticancer taxane contents.[17] In addition to paclitaxel, 10-DAB III, BAC III, and cephalomannine were isolated from hazelnut HSs, GLCs, and leaves.[18-20,24] As an alternative to taxol, the synthetic analog, taxotere™ (generic name Docetaxel), was acquired by semi-synthesis from BAC III and 10-DAB III, two taxol precursor molecules. The American Food and Drug Administration authorized the use of Paclitaxel and Docetaxel for treating ovarian and breast cancers.[25] On the other hand, the natural congener of paclitaxel, cephalomannine has shown anti-tumor activity in several different cell lines.[26] The cytotoxic effects of cephalomannine were first demonstrated in human glioblastoma cells.[27] Later on, Zhang et al.[28] demonstrated that cephalomannine significantly attenuates hepatocellular carcinoma progression *in vitro* and *in vivo* and bone metastasis in prostate cancer *in vivo*.[29] Therefore, cephalomannine has recently been evaluated as an anti-cancer drug candidate.[28-32]

Among Turkish hazelnut (*C. avellana* L.) genotypes (Tombul, Palaz, Çakıldak, Sivri, Kalınkara, Ham, and Sivri Yağlı) used in this study, Tombul, Palaz and Çakıldak categorized as standard and are most grown in Türkiye. However, the cultivation of Tombul and Palaz decreased because they start to leaf early in the spring, while the cultivation of the Çakıldak has increased because it leaves late and is, therefore, more resistant to spring frost. Sivri and Kalınkara, which are standard genotypes, are generally preferred as pollinators due to their lower nut quality. Local genotypes, Ham and Sivri Yağlı, are available only as pollinators in the orchard. Within the 18 cultivars grown in Türkiye, the Tombul is considered the first-quality hazelnut due to its high oil content, distinctive taste, and aroma, and easily and quickly removable BS during roasting.[33] Since it, beings, the most consumed cultivar both nationally and internationally, Tombul hazelnut leaves, HSs and GLCs have been studied for their taxane contents.[6,19] In addition to Tombul, the present work is aimed to investigate the taxane content of other hazels grown in Türkiye, in terms of genotype and hazelnut parts for the first time. Thus, taxane contents of 7 different hazelnuts (Çakıldak, Sivri, Tombul, Palaz, and Kalınkara as standard and Ham, Sivri Yağlı local genotypes) parts [GLCs, HSs, skinless kernels (SKs), BSs, and BSKs] were analyzed by high-performance liquid chromatography (HPLC). Results were discussed from the perspective of functional foods and promising resources for the pharmaceutical industry.

MATERIALS AND METHODS

Plant materials and chemicals

In this study, GLCs and HSs (non-edible parts), SKs, BSs, and BSKs (edible parts) of Çakıldak, Sivri, Tombul, Palaz, and Kalınkara as standard and Ham, and Sivri Yağlı as local hazelnut (*C. avellana L.)* genotypes were used. Tombul, Palaz, and Çakıldak were selected as they were the most grown in Türkiye. The others are preferred as pollinators, but they have not yet been studied. Hazelnut samples were harvested in August 2017 in the Fatsa district of Ordu province (40°59'27.9"N 37°35'01.6"E), Türkiye.The samples were kept in cloth bags at room temperature until analyses were carried out.

HPLC grade of acetonitrile (› 99.93%), methanol (99.99%), and hexane for organic trace analysis UniSolv® were obtained from Merck (Darmstadt, Germany). All the standard reference compounds; 10-DAB III (› 95.0%), BAC III (› 95.0%), cephalomannine (› 97.0%), and paclitaxel (› 95.0%) were acquired from Sigma-Aldrich (Germany).

Taxane extraction

Oil and methanol extractions were adapted from Oguzkan et al.[21] and performed as follows. Five parts (GLCs, HSs, BSKs, SKs, and BSs,) of 7 different Turkish hazelnuts were used as

experimental material. HSs, BSKs SKs, and BSs were separately grounded by the Sinbo SCM 2934 coffee grinder while GLCs were grounded by the Loyka LKD 100 Sample Grinder Mill. GLCs, HSs, BSKs, and SKs were ground to a size of 10 g while BSs were 0.12 g. Then these parts were defatted with hexane in volumetric beakers at room temperature for 1 hour using a magnetic stirrer with a heater (25 °C, 400 rpm). Grounded GLCs, HSs, BSKs, and SKs were defatted with 20.0 mL hexane (1:20, *w/v*). Since the amount of ground BSs was small, the oil was removed with 10 mL of hexane (1:10, *w/v*). Solid-liquid extraction was performed by mixing the grounded samples with 99% methanol on a magnetic stirrer with a heater (25 °C, 400 rpm). The defatted GLCs, HSs, BSKs, and SKs of hazelnuts were extracted by 100.0 mL methanol (1:10, *w/v*) while the defatted BSs were extracted by 10.0 mL methanol (1:10, *w/v*) at room temperature for 24 hours. The resulting slurries were filtered through filter papers into the Erlenmeyer flasks. The solvent was then removed under vacuum at 40 °C at 300 rpm using a rotary evaporator (Heidolph™ Hei-Vap™). The residues were dissolved with 3.0 mL (BSs with 2.0 mL) methanol, and samples were stored at -20 °C in a freezer between analyses.

Sample preparation and HPLC-photo diode array (PDA) analysis of taxane diterpenoid

In this study, 10-DAB-III, BAC-III, cephalomannine, and paclitaxel, were used as standards for taxane analysis. All of the samples were filtered and brought to the condition to be analyzed by HPLC. Analyses were performed on Shimadzu CBM-20A/CBM-20A lite brand HPLC. OVA Spher 100 Phenyl-Hexyl C18 column (4.6 mm x 250 mm, 5 µm particle size) was used as a column. Mobile phase acetonitrile:water gradient system was adjusted. PDA was used as a detector.

Identification of taxane diterpenoids in hazelnut extracts was performed according to Oguzkan et al.[21] The samples were filtered through a 0.20 µm membrane filter into the new amber glass vials (1.5 mL) before being measured in HPLC. The resultant supernatants were pooled and analyzed. The taxane contents in the extracts of hazelnut were quantified by HPLC reverse phase (RP) system (the Shimadzu CBM-20A/CBM-20A lite) having NOVA Spher 100 Phenyl-Hexyl C18 column (4.6 mm x 250 mm, 5 µm particle size). 10-DAB III, BAC III, cephalomannine, and paclitaxel in hazelnut extracts were eluted with a linear gradient of acetonitrile and water (25:75-15 minute, 75-25-40 minute) at a flow rate of 1.0 mL/minute for 55 minute. The gradient method was preferred to separate impurities from plant-derived products and proportional changes were made in the gradient method during the analysis period. Acetonitrile and the water phase were preferred because the RP column is very effective in the separation of impurities. Depending on the polarity of the mobile phase, sharp and clear peaks of the sought compounds were obtained with the gradient method.

The injection volume was 20.0 µL and peak values were detected at 228 nm using a ultraviolet detector (PDA, Germany). Samples were injected into HPLC at 20.0 µL. Identification of taxanes was carried out by comparison of keeping times with standards. All analysis was performed in triplicate.

Calibration curves for 10-DAB III, BAC III, cephalomannine, and paclitaxel standards were prepared at different concentrations (50 mg/L; 25 mg/L; 10 mg/L; 5 mg/L; 2,5 mg/L and 1 mg/L). Calibration curves were prepared by plotting the peak area against the different standard concentrations. In the study for each hazelnut fraction, the standard solutions were first read and the standard curve was drawn. These different concentrations were analyzed by HPLC and retention times of 10-DAB III, BAC III, cephalomannine, and paclitaxel were determined. Chromatograms were monitored at 11-12 minute for 10-DAB III,16-17 min for BAC III, 25-26 minute for cephalomannine, and 26-27 minute for paclitaxel and were given in Figure 1.[33]

All environmental and personal safety precautions were taken during the experimental and analysis procedures. Protective equipment such as gloves, goggles, and aprons was used during these procedures. In the evaporation process, the pressure setting was controlled from the barometer.

Statistical analysis

All the tests were performed with three replicates. The results for each taxane of seven genotypes and their parts are presented as the mean ± standard deviation (SD, n= 3) by Microsoft Office Excel, 2016 Version16.71. Data were analyzed by One-Way analysis of variance (ANOVA) by using Jamovi Version 2.3. Five parts from seven hazelnuts were performed for each taxane. Statistical significance in mean taxane concentrations between genotypes and their parts was evaluated by One-Way (ANOVA) followed by Tukey's and Bonferroni correction correction as a post hoc-test for multiple comparisons in our analysis. These helped ensure the reliability of our statistical inferences. A p value less than 0.01 was considered statistically significant. The graphs were generated by Microsoft Office Excel, 2016 Version16.71. The bars of graphics were calculated as ± SD of the mean for each and total taxanes.

RESULTS

Herewith we report anticancer taxane contents of 5 standards and 2 local Turkish hazelnut genotypes both in their non-edible and edible parts for the first time. In addition to the total taxane contents of hazelnut genotypes (Figure 2), 10-DAB III, BAC III, cephalomannine, and paclitaxel contents are given separately for each hazelnut parts (GLC, HS, SK, BS, and BSK) in Figure

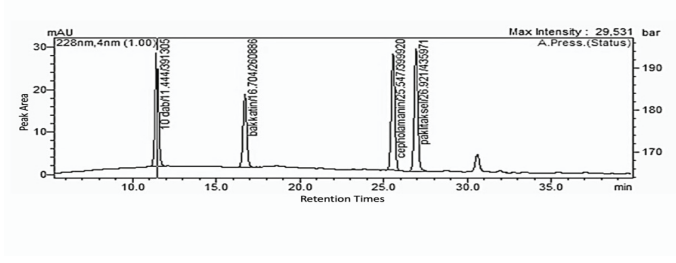

Figure 1. The chromatogram shows the peak areas and retention times of the taxane. 10-DAB III, 11,444 minute and peak area 391,305; BAC-III, 16,704 minute and peak area 260,886; cephalomannine, 25,547 minute and peak area 399,920; Paclitaxel, 26,921 minute and peak area 435,971

10-DAB III: 10-deacetylbaccatin III, BAC-III: Baccatin III

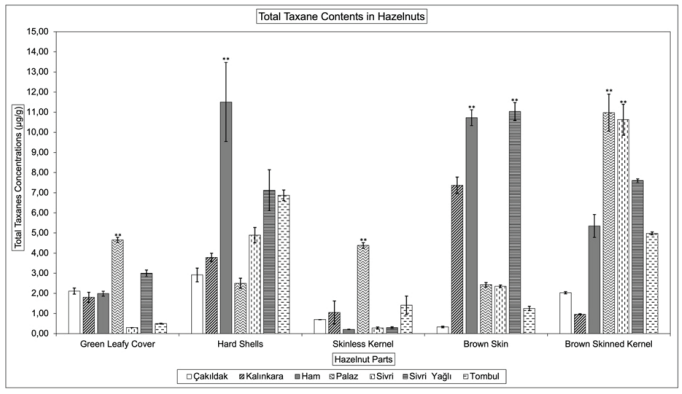

Figure 2. Total taxane contents in GLC, HS, SK, BS, and BSK of seven hazelnut genotypes. Statistical significance between hazelnut genotypes and their parts was determined using One-Way analysis of variance. $^{**}p < 0.01$, demonstrating significant differences in total taxane concentrations among hazelnut genotypes within each region. Bars represent the mean ± SD

GLC: Green leafy cover, HS: Hard shell, SK: Skinless kernel, BS: Brown-skin, BSK: Brown-skinned kernel, SD: Standard deviation

3-7. Concentrations of 10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane from GLC, HS, SK, BS, and BSK of seven hazelnut genotypes (Tombul, Palaz, Çakıldak, Sivri, Kalınkara, Ham and Sivri Yağlı) are given in Table 1.

Total taxane patterns in hazelnut parts of different genotypes

The distribution of total taxanes in hazelnut parts (GLC, HS, SK, BS, and BSK) for seven genotypes is given in Figure 2. Although total taxane profiles of hazelnut genotypes were found to be in a wide range (0.21 and 11.51 µg/g) in tested materials, average taxane contents of the hazelnut parts showed the following trend: BSK (6.08 µg/g) › HS (5.66 µg/g) › BS (5.07 µg/g) › GLC (2.05 µg/g) › SK (1.19 µg/g). Genotypes were ranked according to their total taxane contents in all parts as follows; Ham (29.78 µg/g), Sivri Yağlı (29.06 µg/g), Palaz (24.95 µg/g), Sivri (18.45 µg/g), Tombul (15.01 µg/g), Kalınkara (14.96 µg/g), Çakıldak (8.09 µg/g).

10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane content of green hazelnut leafy cover

10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane contents in the GLC of seven genotypes are given in Figure 3. HPLC results showed that extracts of GLCs mainly contained paclitaxel. The total amount of paclitaxel (7.46 µg/g) comprised 52% of total GLC's taxanes. Although this compound is substantially represented by Palaz, paclitaxel is found in nearly all tested genotypes. 10-DAB III was the second most abundant taxane and comprised 23% of total GLC's taxanes. On the other hand, cephalomannine is not detected and/or in small quantities for GLC extracts.

10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane content of hard HS

10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane contents in the HSs of the seven genotypes are illustrated in Figure 4. In the results obtained, total 10-DAB III (31.06 µg/g), an important intermediate for Taxol®, comprised 78% of total HS's taxane. BAC III was the second most abundant taxane and comprised 14% of total HS's taxane. The HSs of the seven genotypes nearly contained all taxane in their extracts and were the most advantageous parts for these molecules after BSKs.

10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane contents of hazelnut SK

10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane contents in skinless hazelnut kernels of the seven genotypes are presented in Figure 5. The HPLC results showed that the extracts of SKs mainly contained BAC III. The total amount of BAC III (5.67 µg/g) comprised 68% of total SK's taxane. Although this compound is substantially represented by Palaz, BAC III was found in all tested genotypes. Cephalomannine was the second most abundant taxane, constituting 31% of all SK taxanes. On the other hand, 10-DAB III and paclitaxel are not detected or are present in small quantities in the SK extracts.

10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane contents of hazelnut BS

10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane contents in kernel's BSs of seven genotypes are illustrated in Figure 6. In the results obtained, the taxane compound distribution of BS extracts showed parallelism with SKs. BAC III and cephalomannine contents comprised 68% and 32% of total BSs taxanes, respectively. 10-DAB III and paclitaxel were not detected in BS extracts for any tested hazelnut genotypes. However, BS extracts accumulated ~4-fold higher content than SKs for both BAC III and cephalomannine.

10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane content of hazelnut BSKs

10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane contents in BSKs of seven varieties are given in Figure 7. Our results show that BSK extracts showed significant differences from BS and SK extracts in distribution and quantity for taxane compounds. The BSK extracts represented the hazelnut part with the highest abundance of taxanes. While, BS and SK accumulated only BAC III and cephalomannine, nearly all taxanes were identified in BSK extracts. According to our results, extracts of BSK mainly contained cephalomannine (42%). BAC III, the main compound of BS and SK, was the second most abundant molecule in BSKs (32%). Despite 10-DAB III and paclitaxel molecules were not identified in both SK and BS, BSK extracts contained these taxanes at 13% and 14%, respectively.

DISCUSSION

Hazelnut kernels are a nutritious and health-promoting food because of their high content of sanitary lipids, vitamins, essential amino acids, dietary fibers, and specialized phytochemicals that own respectable biological activities. The health benefits of hazelnuts have taken a new significance after they were determined to produce anticancer taxanes.[1,34-36] Although antioxidant and phenolic compounds mainly represent phytochemicals in kernels, findings of paclitaxel and other taxanes also in them, generate a distinguished aspect of health-

Table 1. Concentrations of 10-DAB III, BAC III, cephalomannine, paclitaxel, and total taxane from GLC, HS, SK, BS, and BSK of seven hazelnut genotypes (Tombul, Palaz, Çakıldak, Sivri, Kalınkara, Ham and Sivri Yağlı)

H. parts	H. varieties	10-deacetyl BACIII (µg/g)	BAC III (µg/g)	Cephalomannine (µg/g)	Paclitaxel (µg/g)	Total (µg/g)
GLC	Çakıldak	ND	2.12 ± 0.15	ND	ND	2.12 ± 0.15
	Kalınkara	ND	ND	ND	1.80 ± 0.25	1.80 ± 0.25
	Ham	ND	ND	ND	1.99 ± 0.12	1.99 ± 0.12
	Palaz	1.29 ± 0.08	ND	ND	3.37 ±0.20	4.66 ± 0.13
	Sivri	ND	ND	ND	0.30 ± 0.00	0.30 ± 0.00
	Sivri Yağlı	1.96 ± 0.11	ND	1.04 ± 0.05	ND	3.00 ± 0.16
	Tombul	ND	0.35 ± 0.02	0.14 ± 0.01	ND	0.50 ± 0.02
HS	Çakıldak	2.26 ± 0.38	0.25 ± 0.05	0.40 ± 0.02	ND	2.91 ± 0.34
	Kalınkara	2.73 ± 0.27	0.54 ± 0.01	0.50 ± 0.05	0.02 ± 0.02	3.78 ± 0.21
	Ham	9.15 ± 1.98	1.31 ± 0.14	1.05 ± 0.03	ND	11.51 ± 1.96
	Palaz	2.13 ± 0.03	ND	0.33 ± 0.21	0.04 ± 0.00	2.50 ± 0.25
	Sivri	4.75 ± 0.37	ND	0.11 ± 0.02	0.03 ± 0.03	4.89 ± 0.38
	Sivri Yağlı	4.27 ± 0.32	2.47 ± 0.84	0.37 ± 0.21	0.02 ± 0.01	7.12 ± 1.02
	Tombul	5.77 ± 0.12	1.02 ± 0.15	0.07 ± 0.03	0.02 ± 0.00	6.87 ± 0.26
BS	Çakıldak	ND	ND	0.33 ± 0.04	ND	0.33 ± 0.04
	Kalınkara	ND	7.24 ± 0.43	0.13 ± 0.01	ND	7.37 ± 0.41
	Ham	ND	6.21 ± 0.38	4.52 ± 0.02	ND	10.73 ± 0.39
	Palaz	ND	2.43 ± 0.11	ND	ND	2.43 ± 0.11
	Sivri	ND	2.35 ± 0.06	ND	ND	2.35 ± 0.06
	Sivri Yağlı	ND	4.67 ± 0.41	6.37 ± 0.05	ND	11.04 ± 0.44
	Tombul	ND	1.25 ± 0.11	ND	ND	1.25 ± 0.11
SK	Çakıldak	ND	0.38 ± 0.07	0.32 ± 0.06	0.00	0.70 ± 0.03
	Kalınkara	ND	0.31 ± 0.00	0.73 ± 0.58	0.05 ± 0.00	1.05 ± 0.57
	Ham	ND	0.09 ± 0.00	0.13 ± 0.00	ND	0.21 ± 0.01
	Palaz	ND	3.71 ± 0.09	0.67 ± 0.06	ND	4.38 ± 0.14
	Sivri	ND	0.18 ± 0.00	0.10 ± 0.05	ND	0.28 ± 0.05
	Sivri Yağlı	ND	0.17 ± 0.02	0.11 ± 0.02	0.02 ± 0.00	0.30 ± 0.04
	Tombul	ND	0.83 ± 0.04	0.50 ± 0.40	0.07 ± 0.03	1.41 ± 0.46
BSK	Çakıldak	1.36 ± 0.08	ND	0.67 ± 0.06	ND	2.03 ± 0.05
	Kalınkara	0.24 ± 0.06	ND	0.72 ± 0.05	ND	0.96 ± 0.02
	Ham	0.99 ± 0.47	ND	ND	4.36 ± 0.16	5.35 ± 0.57
	Palaz	0.17 ± 0.02	5.78 ± 1.48	4.74 ± 0.78	0.29 ± 0.03	10.98 ± 0.92
	Sivri	0.42 ± 0.09	4.45 ± 0.72	5.14 ± 0.16	0.62 ± 0.06	10.63 ± 0.77
	Sivri Yağlı	2.26 ± 0.15	ND	5.34 ± 0.21	ND	7.68 ± 0.08
	Tombul	ND	3.22 ± 0.02	1.16 ± 0.02	0.60 ± 0.01	4.98 ± 0.07

All concentrations of taxane are presented as mean ± SD (µg/g dry weight)
10-DAB III: 10-deacetylbaccatin III, BAC-III: Baccatin III, GLC: Green leafy cover, HS: Hard shell, SK: Skinless kernel, BS: Brown-skin, BSK: Brown-skinned kernel, SD: Standard deviation, ND: Not peak was detected, H.: Hazelnut

promoting properties of this nut.[1,17-19,37] Together with kernel bioactivity studies, by-products (GLC, HSs, and BS) have been forwarded to significant research areas for phytochemicals.[35,36] The health benefits of hazelnuts expanded after they were determined to produce anticancer taxanes. Previous works concentrated on the recovery of paclitaxel from by-products of a few hazelnuts (Tombul, Ventimiglia, Genova, Alessandria, Gasaway), and little is known about whether non-edible and

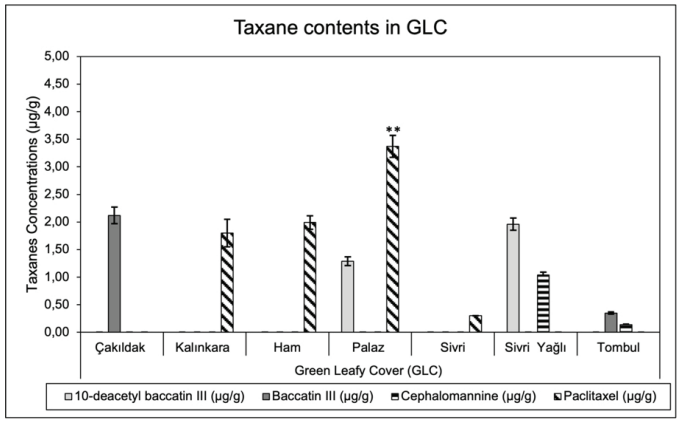

Figure 3. Concentrations of 10-DAB III, BAC III, cephalomannine, and paclitaxel from the GLC. Statistical significance between hazelnut genotypes was determined using a One-Way analysis of variance. **Indicates a p < value 0.01, demonstrating significant differences in the highest taxane concentration among the hazelnut genotypes and their parts. Bars represent the mean ± SD

10-DAB III: 10-deacetylbaccatin III, BAC-III: Baccatin III, GLC: Green leafy cover, SD: Standard deviation

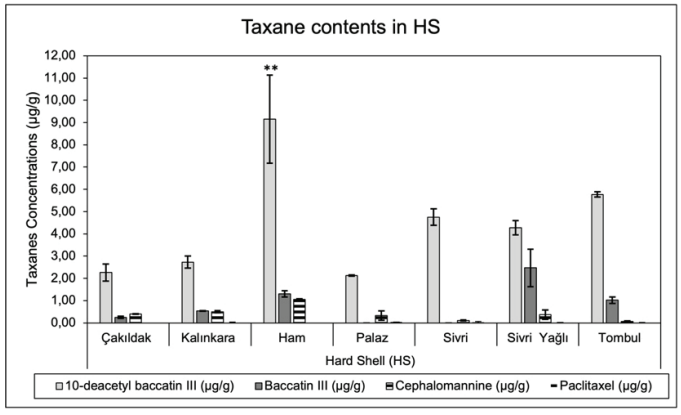

Figure 4. Concentrations of 10-DAB III, BAC III, cephalomannine, and paclitaxel from HS. Statistical significance between hazelnut genotypes was determined using a One-Way analysis of variance. **Indicates a p < 0.01, demonstrating significant differences in the highest taxane concentration among the hazelnut genotypes and their parts. Bars represent the mean ± SD

10-DAB III: 10-deacetylbaccatin III, BAC-III: Baccatin III, HS: Hard shell, SD: Standard deviation

edible parts contained taxanes in Turkish cultivars.[17-19,37]

This is the first report to analyze seven Turkish genotypes separately in terms of paclitaxel and its derivatives and evidenced taxane compounds reveal a high variability depending on the non-edible and edible parts of genotypes. This variability was particularly noticeable in terms of the total taxane content of the genotypes. While Palaz was detected as the most advantageous genotype for GLC (2.3 times higher than average), SK (3.7 times higher than average), and BSK (1.8 times higher than average) extracts, Ham (2 times higher than average) and Sivri Yağlı (2.2 times higher than average) had highest total taxanes of their HSs and BSs, respectively. According to the highest taxane contents, parts of Sivri, Kalınkara, and Çakıldak were ranked as BSK, BS, and HS. Our analysis showed Ham's HS has the

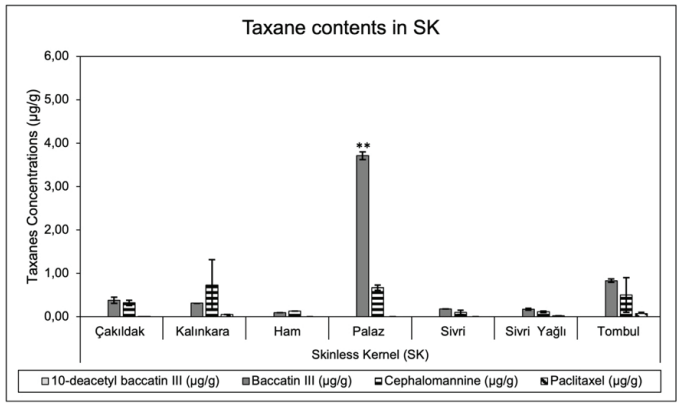

Figure 5. Concentrations of 10-DAB III, BAC III, cephalomannine, and paclitaxel from the SK. Statistical significance between hazelnut genotypes was determined using a One-Way analysis of variance. **Indicates a p < 0.01, demonstrating significant differences in the highest taxane concentration among the hazelnut genotypes and their parts. Bars represent the mean ± SD

10-DAB III: 10-deacetylbaccatin III, BAC-III: Baccatin III, SK: Skinless kernel, SD: Standard deviation

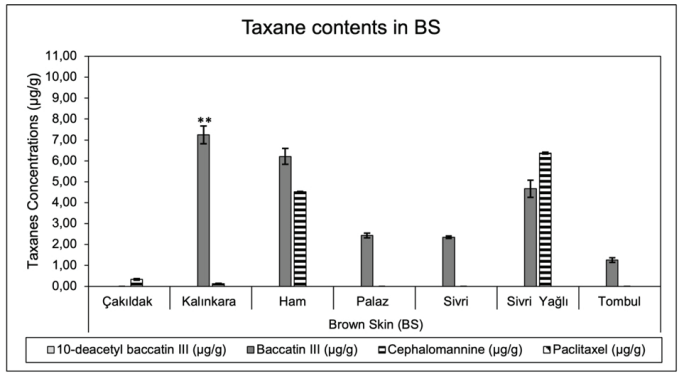

Figure 6. Concentrations of 10-DAB III, BAC III, cephalomannine, and paclitaxel from BS. Statistical significance between hazelnut genotypes was determined using a One-Way analysis of variance. **Indicates a p < 0.01, demonstrating significant differences in the highest taxane concentration among the hazelnut genotypes and their parts. Bars represent the mean ± SD

10-DAB III: 10-deacetylbaccatin III, BAC-III: Baccatin III, BS: Brown-skin, SD: Standard deviation

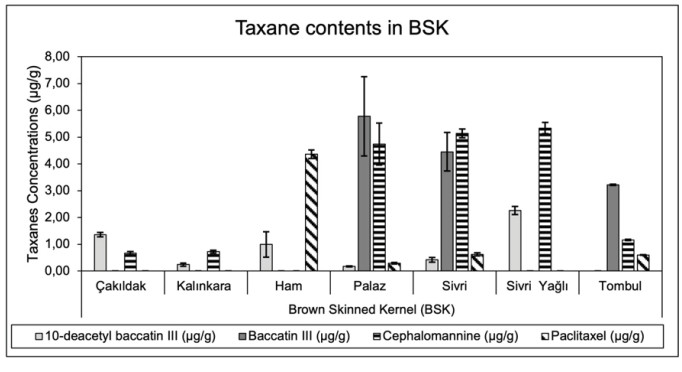

Figure 7. Concentrations of 10-DAB III, BAC III, cephalomannine, and paclitaxel from BSK. Statistical significance between hazelnut genotypes was determined using One-Way analysis of variance. Bars represent the mean ± SD

10-DAB III: 10-deacetylbaccatin III, BAC-III: Baccatin III, BSK: Brown-skinned kernel, SD: Standard deviation

highest total taxane content in all tested parts of genotypes. While the total taxane amount was significantly high in GLC and SK parts of Palaz, there was no difference when compared with Sivri for BSK. HSs of Ham had the highest total taxane content, however, in BS's there was no significant difference between with Sivri Yağlı. Moreover, Tombul, the most known hazelnut genotype in paclitaxel recovery studies, had relatively lower amounts of these compounds (Figure 2).

Since Hoffman et al.[17] discovered paclitaxel in *C. avellana*, HSs have become a prominent material for the investigation of paclitaxel and other taxanes. Hoffman and Shahidi[19] showed HSs contain more taxanes than GLC extracts. Oguzkan et al.[21] analyzed a mix of HS samples collected from different regions of Türkiye and they identified paclitaxel and its precursor BAC III. In the same study, GLCs were also investigated, and BAC III was to be the main taxane. Our analysis indicated that the highest level of total 10-DAB III content among all tested parts was found in the HSs of the hazelnuts. According to our data, GLC extracts contain the highest paclitaxel level in all tested parts. Furthermore, our analysis identifies Palaz as the richest genotype in paclitaxel content among the others. Interestingly, the GLC extracts from the Tombul are similar to the findings of a previous study conducted by Hoffman and Shahidi[19], which proposed that the GLC included only BAC III. These findings highlight the differences in the amount of paclitaxel present in various genotypes and their parts. Taxane contents are affected by multiple variables such as extraction methods, genotype, and growing conditions.[37] Although, these by-products are readily available natural resources for paclitaxel and its derivatives, because of the production of HSs and GLCs in thousands of tons each year.

Despite the kernels not being analyzed in detail, the current study indicated the presence of taxanes in the skinless kernel, its BS, and BSKs of Turkish hazelnut genotypes.[19,37] Among the analyzed hazelnut extracts, the BSK was the most advantageous part for the synthesis of taxane compounds. Together with the diversity present in the genotypes, BSKs contain almost all taxane compounds, and the second most abundant paclitaxel, which is mainly represented by the Ham genotype. Previous research indicated that BSK extracts were unfavorable for Tombul and there is not any knowledge of Ham for the recovery of taxane compounds. The SK extracts were found to have relatively lower taxane production compared to other parts.[19] Previous research emphasized that SK has a general trend showing a lower bioactive potential that is mostly contributed by phenolic compounds.[1] According to our results, skins of the kernels were discovered as the most accumulative part of the kernel for BAC III, an essential precursor compound for the semi-synthesis of Taxol® and a potent anticancer agent cephalomannine. This finding has significant implications for the fields of phytochemical and pharmaceutical research of hazelnuts. It highlights the significance that hazelnut skins are to the bioavailability of these beneficial anticancer compounds and, provides the possible relevance of this natural source in the production of pharmaceuticals.[32] It is known that taxanes such as paclitaxel, which exhibit certain cytotoxicity, prevent cancer cells from proliferating and inhibit the rate at which middle- and late-stage cells transform, are found in hazelnut shells, GLC, and leaves.[5] In addition, recent studies that investigated the activity of cephalomannine in cell viability considered that this compound is a promising natural agent for treating different cancer types.[32] It has also been proven that cephalomannine significantly attenuates hepatocellular carcinoma progression.[28] Li and Parry[38] monitored the antioxidant activities and antiproliferative effects of Turkish and Oregon hazelnut parts and demonstrated that extracts from roasted hazelnut skins suppressed the proliferation of cancer cells and exhibited free radical scavenging properties. Consuming the kernel with BS is strongly suggested for preventing the loss of bioactive compounds from the kernel, although there is no unanimous opinion about the kernel and its BS relationships in terms of bioactivity.[1,38,39]

This is the first report that revealed the differences in taxane contents of Turkish standard and local hazelnut genotypes in different edible and non-edible kernel parts. The data reveals that there are significant differences between genotype and hazelnut parts. Previous studies have demonstrated that the synthesis of taxane compounds in these plants can exhibit significant heterogeneity depending on conditions such as land compositions, height, climate, genotype, and tissue type. Türkiye with a wide-ranging collection of hazelnut genotypes and a leading position in global production has promising potential for the recovery of these compounds and a rich source of this functional food with an anticancer perspective.

Study limitations

In this study, 4 anticancer taxane compounds were analyzed separately in 5 different kernel parts of 7 Turkish hazelnut genotypes and were presented with comprehensive and detailed data for future research. However, in addition to the hazelnut parts analyzed in this study, enriching the results by analyzing leaf and stem samples, in the same way, may contribute to the overall assessment of taxane contents in Turkish hazelnut varieties.

CONCLUSION

Türkiye is a leading global hazelnut producer, resulting in being one of the countries with the highest amounts of by-products such as GLC and HSs. Identification of taxanes in hazelnut by-products which are commonly considered as discarded material and produced thousands of tonnes per year is a future alternative for obtaining these compounds from readily available natural resources. Moreover, our findings challenged previous knowledge about the recovery of taxanes from edible parts of hazelnuts. While HSs, BSKs, and BSs were rich in taxanes in all of the analyzed hazelnut genotypes, SKs, and GLCs remain limited for these anticancer taxanes. Surprisingly, BSKs of local genotypes were found rich in taxanes than skinless kernels of standard genotypes. Our study has revealed significant findings on the abundance of taxane compounds in Turkish hazelnuts including previously untested genotypes and their parts. These findings are expected to highlight the

health benefits of consuming raw Turkish hazelnuts with BSs and their possible use as a functional food. Perhaps regular consumption of a certain amount of the kernels of Ham, Palaz, Kalınkara, Sivri, and Tombul hazelnuts containing the paclitaxel compound can prevent the irregular proliferation of body cells. Collectively, the data presented in this study can be used not only in breeding projects aiming at choosing cultivars with higher levels of health-promoting compounds but also to expand the pharmaceutical potential of local genotypes with further studies based on using a holistic approach.

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Ethics

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Authorship Contributions

Concept: Ş.A., Design: G.Z.K., E.S.D., B.K., H.İ.U., Ş.A., Data Collection or Processing: G.Z.K., E.S.D., B.K., Analysis or Interpretation: G.Z.K., B.Y., Literature Search: B.Y., H.İ.U., Ü.S., Ş.A., Writing: G.Z.K., B.Y., Ş.A.

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