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ORIGINAL RESEARCH PAPER

EXPLORING THE NUTRITIONAL BENEFITS OF ACORNS: PHYTOCHEMICAL PROFILES AND BIOLOGICAL ACTIVITY

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Abstract

Acorn, the fruit of *Quercus* species, is an edible nut with a long history in human diet and food resources. The study aimed to investigate the nutritional profiles of kernels and pericarps of acorns from *Q. castaneifolia* (*Qc*) and *Q. brantii* (*Qb*). Analysis of the Qc kernel revealed a high starch content (18.7±1.5%) and a lipid fraction rich in oleic acid (42.0%) and linoleic acid (34.1%). The *Qb* acorns exhibited a high quantity of total phenols (386.7 mg g^{-1} as gallic acid equivalent) and protein (1.30±0.19 mg g^{-1}). Phytochemical analysis of the pericarp from *Qc* revealed a great flavonoid content (146.4 mg g^{-1} as catechin equivalent), contributing to its excellent antioxidant activity, as demonstrated by ABTS assays. The essential oil of the kernels from both acorn species contained *α*-pinene, heptanal, *β*-myrcene, limonene, methyl hexadecanoate, 2,5-Bis(1,1-dimethylethyl) phenol, 2,6-dimethyl naphthalene, and *n*-nonanal. The extract of *Qc* acorns exhibited inhibitory effects on the growth of *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and *E. coli* as common pathogenic bacteria. With its rich compositional profiles and potent antioxidant and antimicrobial properties, acorn shows great promise in nutraceutical and nutritional benefits.

Keywords: acorn, oak fruit, phytochemical composition, *Quercus castaneifolia; Q. brantii*

Introduction

The genus *Quercus* from the family *Fagaceae* includes approximately 450 species worldwide that grow as shrubs or trees in various regions of Asia, Europe, America, and North Africa (Vinha *et al*., 2016). The oak trees are mainly used in diverse

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applications, *e.g.*, furniture manufacturing, fuelwood, animal feeding, soil protection from erosion, and also in folk medicine as astringents, antidiarrhea, antiulcer, antiinflammation, *etc* (Özcan 2006). *Quercus* species yield a universally known fruit, identified as acorn, a nut with a single seed, surrounded by a hard and durable shell, which has been utilized as food for humans and animals over the centuries. Acorn is highly important for Americans and Europeans, which provides part of their diet and comprises one of the feeding sources for domestic animals such as Iberian pigs and cattle (Cantos *et al*., 2003). It is also used for tannin extraction which has historically been used in various industries, *e.g.*, leather tanning, textile dyeing, and ink production. Acorn is applied in some Mediterranean countries instead of nuts or dried fruits. Likewise, acorn flour is commonly utilized to make local bread or cake in areas where oaks are available. Besides its folkloric uses as a food ingredient, in several regions, the roasted acorn is utilized as herbal tea (Silva *et al*., 2016; Vinha *et al*., 2016). Furthermore, *in vitro* studies have shown that the shells of acorns possess angiogenesis activity and have traditionally been employed as a natural remedy for promoting wound healing (Vinha *et al*., 2016).

Acorns are considered nutritionally rich products, containing a high amount of starch (*ca.* 55%), which makes them a suitable alternative to high starch content products such as potatoes and chestnuts (Saffarzadeh, *et al*., 1999; Vinha *et al*., 2016), and low amount of protein (2.8–8.4%), and fat (0.7–7.4%) (Özcan 2006; Özcan 2007). Acorns contain significant amounts of minerals, such as potassium, calcium, and magnesium, as well as vitamins, mostly A and E (Vinha *et al*., 2016). Accordingly, nuts display a high nutritional value, comparable with cereals, which can be an essential nutrient in foods containing wheat flour, such as pastries, noodles, muffins, cookies, bread, and desserts. As a result of its nature, it could contribute to preparing pertinent gluten-free products with an ever-growing market (Silva *et al*., 2016). Moreover, acorns are of great interest due to their abundance of phytochemicals. They comprise high phenolic compounds, *e.g.*, gallic acid and ellagic acid derivatives, various flavonoids, volatile organic compounds, sterols, and aliphatic alcohols. These bioactive phytochemicals have been found to provide numerous health benefits, primarily owing to their potent antioxidant activity and, consequently, their ability to reduce the risk of various diseases such as cardiovascular and inflammatory illnesses, diabetes, cancers, microbial infections, human immunodeficiency virus (HIV) infection and other ailments which enable the utilization of acorns in the preparation of functional foods (Vinha *et al*., 2016).

Nevertheless, acorns from different *Quercus* species reveal significant variability in the chemical composition resulting from phylogenetic and ecological aspects. Each species possesses particular characteristics that can impact the functional efficiency of a given food product. Considering the significant potential bioactive compounds in the acorn components, the present study aims to quantify and compare the phytochemicals and biological activities of two *Quercus* species, *Q. brantii* Lindl. and *Q. castaneifolia* C.A. Mey.

Materials and methods

Materials, culture media, and microorganisms

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, and catechin were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Potassium ferricyanide ($K_3Fe(CN)_6$), ferric (III) chloride ($FeCl_3$), boron trifluoride (BF_3), sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium bicarbonate ($NaHCO_3$), magnesium chloride ($MgCl_2$), tris-hydrochloride (Tris-HCl), ethylenediaminetetraacetic acid (EDTA), dithiothreitol (DTT), dimethyl sulfoxide (DMSO), Folin-Ciocalteu reagent and Coomassie Brilliant Blue G-250 were provided by Merck (Darmstadt, Germany). The bacterial strains were *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 9027, and *Escherichia coli* ATCC 8739. Muller Hinton Agar (MHA) was also purchased from Merck (Darmstadt, Germany).

Sample collection

The oak fruits of *Q. castaneifolia* and *Q. brantii* were respectively collected from the forest of Hyrcanian (Mazandaran Province, north of Iran; 36°25'50.4"N 52°17'39.0"E) and the forest of Zagros (Kohgiluyeh and Boyer-Ahmad Province, western of Iran; 30°50'28.8"N 51°28'46.1"E) in October 2022. Plant species were identified by the herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. The acorns of each species were then separately dried, dehulled into kernel and pericarp, and pulverized (Figure 1).

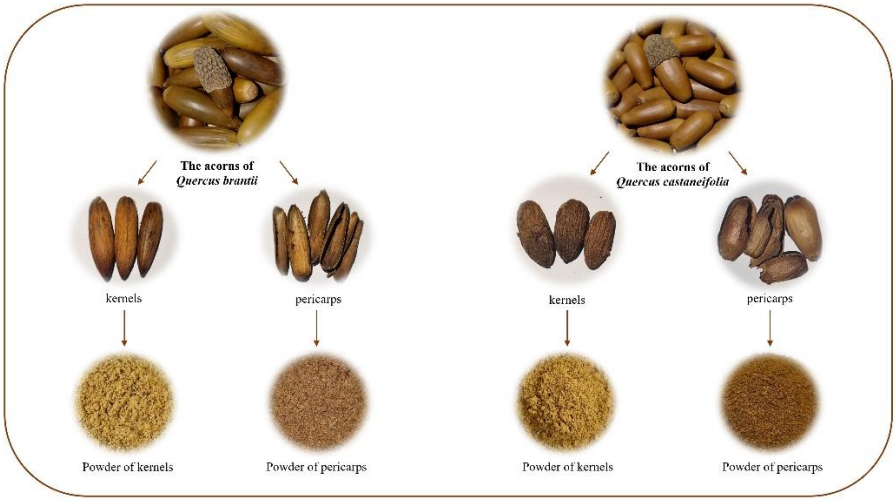

Figure 1. The acorns of *Quercus castaneifolia* (a) and *Q. brantii* (b).

Preparation of the extracts

The extracts of two species of acorns were individually prepared by maceration of 10 g of each kernel and pericarp powders in 200 mL of 70% methanol three times,

each for 24 h, with stirring periodically. Each mixture was then filtered and concentrated under vacuum pressure at 40 ºC using a rotary evaporator. The obtained four dried extracts were then stored in the dark at 4 ºC before use.

Determination of starch content

The powder of the kernel and pericarp of each species (2 g) were separately dispersed in 0.3% sodium hydroxide (10 mL), mixed well, and left to stand for 2 h. Then, the dispersion was filtered through a sieve with a mesh size of 0.21 mm. The solution was allowed to settle and the liquid portion was discarded. The precipitate was rinsed several times with distilled water and air-dried. This entire process was repeated three times for each sample.

Quantification of total protein

The powder of the kernel and pericarp of each species, weighing 1 g, was dispersed in 6 mL 50 mM Tris-HCl, pH 7.8 consisting of 0.5 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM dithiothreitol (DTT). The mixture was centrifuged at 10,000 × *g* at 4 ºC for 20 min. Soluble proteins are present in the supernatant. Then a solution of sodium hydroxide (1 M, 3 mL) was added to the precipitate and incubated at 90 ºC for 20 min. The mixture was then centrifuged at 4,000 × *g* for 10 min at 4 ºC. The non-soluble protein was presented in the supernatant. The extract protein contents were determined by Coomassie Brilliant Blue G-250 according to the method of Bradford and bovine serum albumin was used as the standard. The tests were conducted three times for each sample.

Measurement of the ash

A porcelain crucible containing 5 g of each sample was heated to 550 ºC in an oven for 8 h. It was then transferred to a desiccator and weighed immediately after cooling. The experiments were carried out three times, and total ash was reported as a percentage of dry weight (Equation 1).

$$\text{Ash (w/w \%)} = ((\text{weight of ash (g)}/ \text{weight of test portion (g)}) \times 100 \quad (1)$$

Determination of soluble and insoluble lignin

To determine the soluble and insoluble lignin content, 1 g of each sample was homogenized in 50 mM potassium phosphate buffer (pH 7) using a mortar and pestle. The homogenate was then centrifuged at 5000 *g* for 10 min. The resulting pellet underwent sequential washes with phosphate buffer (twice), 1% (v/v) Triton X-100 in pH 7.0 buffer (three times), 1 M NaCl in pH 7.0 buffer (twice), distilled water (twice), and acetone (twice). The residues were subsequently dried at 100 °C, cooled in a desiccator, and digested with 15 ml of cold 72% sulfuric acid while stirring for 2 h at 20 °C. Following rinsing of the mixture and adjustment of the total volume with water, the solution was diluted to 3% H_2SO_4, refluxed at 80 °C for 4 h, and subsequently filtered to isolate the soluble and insoluble fractions. The absorbance of the filtrate was measured at 280 nm to quantify the acid-soluble lignin, using equation 2:

$$\text{Soluble lignin (g L}^{-1}) = A280/ 110 \quad (2)$$

To calculate the insoluble lignin content, the dry mass weight of each sample was assessed, and the total ash weight was subtracted from this value. The total lignin content was determined by summing the acid-soluble and acid-insoluble lignin components (Moreira-Vilar *et al*., 2014).

Study on the fatty acid profile

Lipid extraction was carried out with *n*-hexane as the organic solvent. For this experiment, 50 g of kernel powder of each species was mixed with 120 mL of *n*-hexane and sonicated for 15 min. The mixture was filtered, and the residue was re-extracted using 100 mL *n*-hexane. The *n*-hexane extract was collected, and the solvent was evaporated. The extracted oil was weighed, and its percentage weight was reported as a percentage of the weight of powdered kernels. Afterward, fatty acid methyl esters (FAME) were synthesized by using the Metcalfe method. In the steam bath, 0.4 g of the fatty extract with 5 mL of 2% methanolic sodium hydroxide was heated for 10 min. After chilling, 1.175 mL of methanolic boron trifluoride (BF_3, 20%) was added, and the mixture was once more heated in the steam bath for 2 min. Then, 1 mL of saturated sodium chloride was added to the mixture and shaken instantly. After separating the two phases from each other, 0.2 µL of the upper phase, which contains FAME, was analyzed using gas chromatography (Unicam 4600, Cambridge, UK) equipped with a flame ionization detector and BPX70 capillary column (0.22 mm *i.d*., 30 m, 0.25 µm film thickness; J & W Scientific, CA, USA). The initial column temperature was held at 140 °C for 5 min, raised at a rate of 20 °C min^{-1}, maintained for 9 min at 180 °C, increased to 200 °C at the same rate, and kept for 25 min. The injector and detector temperatures were set at 250 °C and 300 °C, respectively. The split ratio and sample injection were 1/100 and 0.2 µL, and helium was used as the carrier gas. Quantification of FAME was accomplished by comparing the area under the curve of each peak with those of pure standards (Sigma-Aldrich, St. Louis, MO, USA), and the percentage composition of each fatty acid was determined as the ratio of its mass to the total oil mass, expressed as a percentage.

Total phenolic content

The amount of phenolic compounds in each species was measured using the Folin-Ciocalteu reagent. The kernel and pericarp extract of each species (at a concentration of 1 mg/mL in methanol) was combined with 5 mL of the diluted Folin-Ciocalteu reagent (1:10) and left at room temperature. After 10 min, sodium bicarbonate (75 mg/mL) was added to the mixture, which was then brought up to 10 mL using distilled water. The mixture was incubated in darkness for 30 min, and the absorbance was measured at 765 nm compared to the gallic acid calibration curve (50-200 µg/mL). The tests were conducted three times.

Total flavonoid content

The aluminum chloride colorimetric method (Sánchez-Burgos et al., 2013) was used for the determination of the total flavonoid content of the plant extracts. Briefly, 250 µL of each extract solution (1 mg mL^{-1}) was mixed with 75 µL 5% sodium nitrite followed by 150 µL of a freshly prepared 10% $AlCl_3$, and aqueous 500 µL aqueous

1 M NaOH. To achieve a final volume of 2.5 mL, distilled water was employed for adjustment. The mixture was then incubated at room temperature for 5 min. Subsequently, the absorbance was measured at 510 nm, with a blank used as a reference. The experiments were repeated three times and the calibration curve was standardized using catechin. The concentration of flavonoid in the extract was expressed as mg catechin equivalent per gram of sample (mg g^{-1}) (Sánchez-Burgos *et al*., 2013).

Quantification of tannin content

For measuring the tannin content, 0.1 g of each kernel and pericarp powder was mixed with 50 mL distilled water and boiled for 30 min. The solution was then filtered and transferred to a 500 mL flask, and filled with distilled water up to 500 mL. Afterward, 0.5 mL portions were taken and mixed with 1 mL 1% potassium ferricyanide and 1% ferric (III) chloride followed by the addition of distilled water to achieve a total volume of 10 mL. The mixtures were spectrophotometrically analyzed at a wavelength of 720 nm after 5 min (Paaver *et al*., 2010). The tannin concentrations were determined beaded on catechin standard solutions in the range of 5–25 µg 10 $mL^{-1,}$ and each sample was tested three times.

Gallic acid determination

This analysis was conducted using high-performance liquid chromatography-photodiode array detection (HPLC-PDA) (Knauer, Germany), equipped with the Eurospher II 100-5 C18 column (250 mm × 4.6 mm, 5 µm particle size) at a flow rate of 1 mL min^{-1}. The column temperature was maintained at 25 °C, and the detection wavelength was set at 280 nm. A gradient solvent system consisting of solvent A (2% aqueous acetic acid) and solvent B (acetonitrile) was used as follows: 0–10 min, 0–5% B; 10–30 min, 5–30% B; 30–35 min, 30–100% B; 35–40 min, 100% B. By employing external calibration with gallic acid as the standard, quantification was performed at a wavelength of 280 nm and the obtained results were then calculated concerning the dry matter.

Gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil

The powdered kernels (30 g) of the two species were subjected to hydrodistillation for 3 h, using a Clevenger apparatus. The obtained oils were dried over anhydrous sodium sulfate and kept at 4 °C until use. The essential oils were analyzed using an HP-6890 gas chromatograph equipped with The essential oils were analyzed using an HP-6890 gas chromatograph equipped with an HP-5MS column (0.25 mm i.d., 30 m, 0.25 µm film thickness) and an HP-5973 mass detector (ionization energy: 70 eV). The analysis was conducted under specific conditions, including a temperature program of 60 °C (0–3 min) and a subsequent increase to 280 °C at 3 °C min^{-1} (3–65 min). The injector temperature was set at 240 °C, while the detector temperature was maintained at 250 °C. A volume of 1.0 µL of the sample was injected with a split ratio 1:50. Helium was used as the carrier gas with a flow rate of 1 mL min^{-1}. To identify the compounds in the essential oils, computer matching was performed using the Wiley7n.L and Wiley275.L libraries. Additionally, the compounds were

compared to standard compounds based on Kovats indices (KIs) and mass fragmentation patterns that had been previously published.

Measuring the antioxidant activity

The antioxidant activity of extracts was analyzed employing two methods including 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assays.

DPPH radical assay

The radical-scavenging potential of various antioxidants is commonly assessed using DPPH, a stable free radical. To determine the DPPH radical scavenging activity, serial dilutions of the extract of kernel and pericarp from *Q. castaneifolia* and *Q. brantii* (0.5 to 500 µg mL^{-1} in methanol) were added to 2 mL of methanolic DPPH solution (0.1 mM) and incubated in dark conditions. After 30 min, the absorbance was measured at 517 nm using methanol as a blank, and three runs of the experiment were performed for each sample. The radical scavenging activity was calculated using equation 3; where Ac and As represent the absorbance of the blank and the absorbance of the radical solution mixed with each sample, respectively. The half-maximal inhibitory concentration (IC_{50}) value of each extract was subsequently calculated.

$$\text{Radical scavenging activity (\%)} = [(Ac - As)/ Ac] \times 100 \quad (3)$$

ABTS radical activity

The ABTS radical scavenging activity was assessed by adding each extract at concentrations ranging from 0.01–2 mg mL^{-1} to a 3.9 mL ABTS radical solution. The reaction was allowed for 30 min at room temperature, after which the absorbance at 734 nm was measured. The obtained results were expressed as IC_{50} values representing the concentration of the extract required to scavenge 50% of the ABTS radicals. Each sample was tested three times.

Antibacterial activity

The well-diffusion method was used to evaluate the antibacterial activity of each extract against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and *E. coli* (Silva et al., 2023). Briefly, Mueller-Hinton agar (MHA) plates were inoculated with respective bacteria. The wells of 6 mm diameter were punched in the agar and different concentrations of extracts (6.25–200 mg mL^{-1}) were filled into the wells. Ciprofloxacin and dimethyl sulfoxide (DMSO) were also poured into one well each as a positive and negative control, respectively. Following a 24-hour incubation at 37 ºC, the diameter of the inhibition zones was assessed in mm.

Results and discussion

Starch content

The total starch content of *Q. castaneifolia* kernel, *Q. castaneifolia* pericarp, *Q. brantii* kernel, and its pericarp were 18.7%, 1.9%, 17.6%, and 0.9%, respectively (Table 1). Based on similar studies, starch contents vary across the *Quercus* species,

for instance, it was found to be 17.3%, 34.5%, and 54.7% in three species of *Q. palustris* (Stevenson et al., 2006), *Q. ilex* (Zarroug et al., 2020), and *Q. leucotrichophora* (Soni et al., 1993), respectively. The variation in extraction methods, sieve mesh, and type of species could potentially account for this difference. A comparison of the isolation methods showed that the starch yield of *Q. brantii* var. *Persica* with the wet milling extraction method was 59%, while it was reported 56% by using the enzyme-assisted extraction method, 51% by the ultrasonic-assisted extraction, and 47% by the lactic acid-assisted extraction (Bayati *et al*., 2022). The amount of starch in *Q. brantii* was stated to be 58.8% (Saffarzadeh *et al*., 1999), which is significantly higher than the obtained data from the current study. This indicates that the acorns were harvested during a stage of starch degradation in their physiological growth and development (Stevenson *et al*., 2006).

Table 1. Chemical analysis of the acorn kernel and pericarp of two *Quercus* species, *Q. castaneifolia* and *Q. brantii*. The data were reported as mean ± standard deviation from three separate experiments.

	Q. castaneifolia		***Q. brantii***	
	kernel	**pericarp**	**kernel**	**pericarp**
Starch content (%)	18.7 ± 1.5	1.9 ± 0.1	17.6 ± 1.1	0.9 ± 0.1
Soluble protein (mg g^{-1} dry matter)	0.67 ± 0.08	0.68 ± 0.09	1.30 ± 0.19	1.21 ± 0.44
Non-soluble protein (mg g^{-1} dry matter)	5.54 ± 0.31	7.30 ± 0.50	12.07 ± 0.06	8.52 ± 0.48
Ash content × 10^{-2} (%)	1.8 ± 0.1	1.4 ± 0.1	1.7 ± 0.1	2.1 ± 0.1
Insoluble lignin (klason) (%)	2.2 ± 0.1	28.3 ± 0.3	7.5 ± 0.3	32.6 ± 0.2
Soluble lignin (%)	0.4 ± 0.02	0.9 ±0.05	0.5 ± 0.03	1.1 ± 0.10
Total lignin (%)	2.6 ± 0.1	29.2 ± 0.4	8.0 ± 0.3	33.7 ± 0.5

Protein content

The soluble and non-soluble protein contents of *Q. castaneifolia* and *Q. brantii* acorn kernels and pericarps are shown in Table 1. *Q. brantii* exhibits a greater amount of protein in both its kernel and pericarp in comparison to *Q. castaneifolia*. The protein level of the kernels and pericarps of *Q. ithaburensis*, a *Quercus* species from the Mediterranean region, was determined to be 2.13% and 2.84%, respectively. Meanwhile, in *Q. calliprinos*, another Mediterranean *Quercus* species, the protein content was assessed to be 2.77% in the kernel and 4.94% in the pericarp (Rababah *et al*., 2008). The evaluation of 20 Turkish *Quercus* species showed that the protein content varied from 2.75% in *Q. pontica* to 8.44% in *Q. infectoria* (Özcan, 2006). Furthermore, the comparison of the protein content of *Q. brantii* collected from three climate regions revealed that the lowest (3.7%) and the highest (4.4%) levels were related to tropical and cold climates. Despite significant differences in protein

amounts based on climate, the amino acid content of *Q. brantii* acorns remained consistent (Saffarzadeh *et al*., 1999).

Total ash

Table 1 also displays the amount of ash in the kernels and pericarps of *Q. castaneifolia* and *Q. brantii*. Based on the results, the ash content of *Q. castaneifolia* and *Q. brantii* kernels was similar, while the pericarp of *Q. brantii* displays a significantly higher ash content than that of *Q. castaneifolia*. The mean total ash level in the kernel and pericarp of *Quercus* species was respectively reported as 1.7% and 1.5% (Vinha *et al*., 2016). For *Q. ithaburensis*, the ash content in the kernel and pericarp was determined to be 3.2% and 2.9%, respectively (Rababah et al., 2008). Two Tunisian *Quercus* species, *Q. ilex* and *Q. coccifera*, displayed total ash levels of 2.2% and 2.5%, respectively (Lassoued *et al*., 2022). Furthermore, in a separate investigation, the *Q. brantii* sample exhibited a total ash content of 1.5%, aligning with the findings of the current study (Saffarzadeh *et al*., 1999).

Lignin content

The concentrations of soluble and insoluble lignin in the kernels and pericarps of *Q. castaneifolia and Q. brantii* are presented in Table 1. Based on the data, *Q. brantii* exhibits higher lignin levels in its kernels and pericarps than *Q. castaneifolia*, with a particularly significant increase in pericarp lignin content. For *Q. pubescens*, the lignin content was recorded at 40.7% (Toscano and Cimino, 2013), whereas the insoluble lignin content in the pericarp of *Q. variabilis* was 24.6% (Zhang et al., 2018). These observations indicate species-specific variations in lignin composition within the *Q. genus*. Additionally, the total lignin content in the cupules of *Q. suber*, *Q. ilex*, and *Q. pyrenaica* was found to be 23.6%, 32.9%, and 20.2%, respectively. These findings align closely with the obtained results, demonstrating significantly greater klason lignin (22.9%, 31.8%, 19.2%) relative to soluble lignin (0.7%, 1.1%, 1.0%) in the respective species (Caeiro, 2024).

Fatty acid content

The total oil extracted from *Q. castaneifolia* and *Q. brantii* kernels was 2.16% and 0.45%, respectively. The fatty acid compositions and percentages of *Q. castaneifolia* and *Q. brantii* kernels were analyzed and documented in Figure 2. Oleic acid, linoleic acid, and palmitic acid are the main fatty acids in both species which were in accordance with similar studies, however, a significant variation of these fatty acids content was observed between *Q. castaneifolia* and *Q. brantii*. Oleic acid is the main monounsaturated fatty acid found in high quantities in *Quercus* spp. such as *Q. virginiana* (66%), *Q. coccifera* (66.5%) (Valero-Galván *et al*., 2021), *Q. petraea* (41.4%), and *Q. trojana* (49.7%) (Özcan, 2007). In terms of oleic acid content, acorns surpass some well-known fruits that are typically recognized as natural sources of oleic acids, such as walnuts (21%), mustard oil (36.7%), and peanuts (38.4%) (Maguire *et al*., 2004). The oil of acorn contains a great amount of linoleic acid (from 14% in *Q. ilex* to 49.1% in *Q. hartwissiana*) (Taib *et al*., 2020). It is an essential unsaturated fatty acid in the human diet that cannot be synthesized internally and should be obtained through the diet. An unsaturated fatty acid-rich

diet is effective in the prevention of type 2 diabetes mellitus (Taib *et al*., 2020). Furthermore, monounsaturated fatty acids such as oleic acid and α-linolenic acid, play a crucial role in the synthesis of eicosanoid, promoting the decrease of blood serum triglycerides and the increase of HDL-cholesterol levels. Based on these findings, it can be concluded that acorn oil is a beneficial source of essential fatty acids that can be incorporated for dietary purposes. It should be noted that the fatty acid profiles of oils derived from distinct acorn species disparate greatly in terms of monounsaturated, polyunsaturated, and saturated fatty acids. This variation is influenced by genetic factors, plant species, maturation level, climate, and geographic origin (Taib *et al*., 2020; Vinha *et al*., 2016).

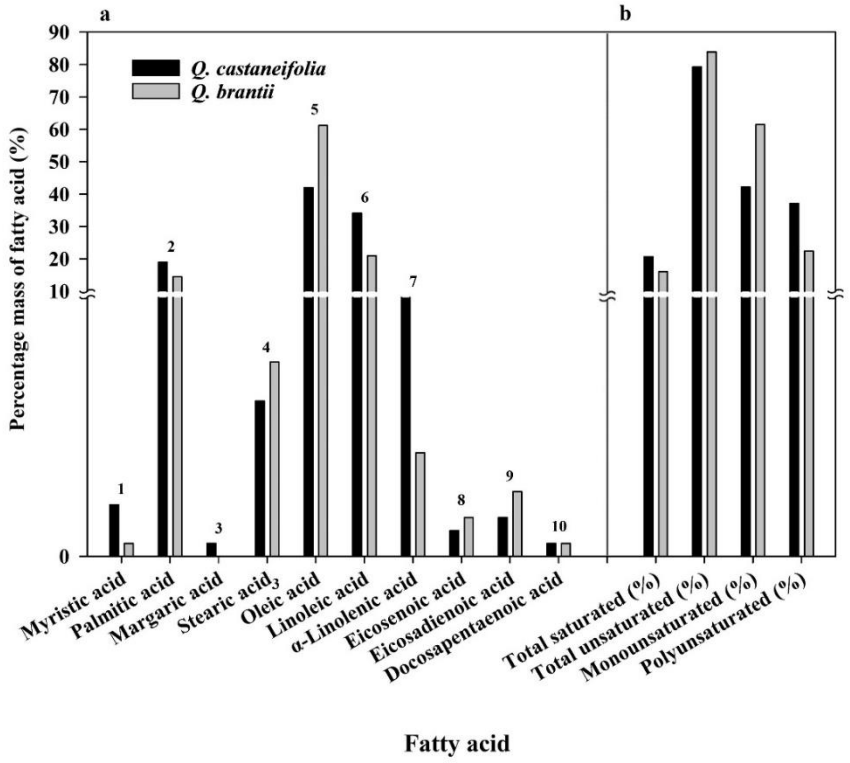

Figure 2. Fatty acid composition (%) of the kernels of *Quercus castaneifolia* and *Q. brantii* (a); total amounts of saturated and unsaturated fatty acid (%) in the kernels of *Q. castaneifolia* and *Q. brantii* (b). The percent mass of each fatty acid was calculated as the ratio of its mass to the total mass of extracted oil.

Total phenolic content

The Folin-Ciocalteu method was employed to determine the total phenolic contents of the acorn kernel and pericarp of *Q. castaneifolia* and *Q. brantii*. The results were expressed as gallic acid equivalent and are presented in Table 2. The amount of total phenolics varied from 153.6 mg gallic acid g^{-1} in the kernel of *Q. brantii* to 386.7

mg gallic acid g^{-1} in the pericarp of *Q. brantii*. As indicated, pericarps displayed a higher total content of phenolics which is consistent with the report of Silva et al (2023) that *Q. suber* and *Q. ilex* pericarps demonstrated total phenolic levels 2.1 and 3.7 times higher than their kernels. However, this is contrary to the study of Cantos et al. (2003), which reported that the acorn kernels exhibited higher phenolic content than their pericarps. This suggests the influence of the variation of geographical and climatic conditions on the level of phenolic compounds in acorns. Despite variations in phenolic levels among various species of *Quercus*, these results suggest that acorns possess significant amounts of phenols. Phenolic compounds have gained significant attention due to their potent antioxidant properties and ability to prevent cardiovascular, neurodegenerative, and other oxidative stress-associated diseases. The phenol content of a natural product is a key indicator of its quality.

Table 2. The contents of total phenol, flavonoid, tannin, and gallic acid of the acorn kernel and pericarp of *Quercus castaneifolia* and *Q. brantii*. Values are expressed as the mean of three replications ± standard deviation.

	Q. castaneifolia		***Q. brantii***	
	kernel	**pericarp**	**kernel**	**pericarp**
Total phenol [a]	181.6 ± 9.0	363.1 ± 8.2	153.6 ± 8.3	386.7 ± 10.5
Total flavonoid [b]	33.8 ± 1.0	146.4 ± 1.2	23.3 ± 1.1	118.4 ± 1.2
Tannin [c]	67.3 ± 0.7	57.2 ± 0.5	52.3 ± 1.1	107.0 ± 3.0
Gallic acid [d]	15.5 ± 0.4	11.6 ± 0.5	5.3 ± 0.2	10.3 ± 0.4

[a] Data expressed in mg equivalent of gallic acid to 1 g of the extract; [b] Data expressed in mg equivalent of catechin to 1 g of the extract; [c] Data expressed in mg equivalent of catechin to 1 g of dry matter; [d] Data expressed

Total flavonoid content

The total flavonoid content of the extracts was spectrophotometrically determined by the aluminum nitrate colorimetric method and expressed as catechin equivalents (mg g^{-1} extract) (Table 2). The highest total flavonoid quantity was observed in the pericarp of *Q. castaneifolia* (146.4 mg catechin g^{-1}), while the kernel of *Q. brantii* exhibited the lowest amount (23.3 mg catechin g^{-1}). The research findings revealed that the pericarp of *Q. castaneifolia* and *Q. brantii* contained a greater quantity of total flavonoids compared to their kernels. Similarly, the kernel of *Q. coccifera* L. showed a lower quantity of flavonoids than its pericarp. However, it is worth noting that the flavonoid content in the kernel of *Q. coccifera* L. was still higher than that observed in the present study, with values of 73.2 and 179.0 mg catechin g^{-1} for the kernel and pericarp, respectively (Gezici and Sekeroglu, 2019). Furthermore, the total flavonoid contents of the kernels of *Q. brantii* and *Q. castaneifolia* were also found to be higher than those determined in acorns from *Q. ilex* (9.2 mg catechin g^{-1}), *Q. suber* (9.2 mg catechin g^{-1}), *Q. virginia* (13.9 mg catechin g^{-1}), and *Q. laeta* (18.9 mg catechin g^{-1}) although it was lower those of *Q. coccifera* (56.3 mg catechin g^{-1}) and *Q. resinosa* (39 mg catechin g^{-1}) (Sánchez-Burgos *et al*., 2013; Valero-Galván *et al*., 2021). Geographical conditions and the type of solvent for extraction

of plant materials are factors that exert an influence on the quantity of flavonoids, similar to the impact observed on total phenol levels, as previously discussed (Gezici and Sekeroglu, 2019).

Tannin content

The tannin content was assessed for both kernel and pericarp of *Q. castaneifolia* and *Q. brantii*. According to Table 2, *Q. brantii* pericarp was found the highest amount of tannin (107 mg catechin g^{-1}) followed by *Q. castaneifolia* kernel, *Q. castaneifolia* pericarp, and *Q. brantii* kernel with 67.3, 57.2, and 52.3 mg catechin g^{-1}, respectively. In comparison, the tannin levels of two other *Quercus* species, *Q. robur* and *Q. cerris*, were determined higher than those obtained in the current study (204 and 218 mg gallic acid g^{-1}) (Rakić *et al*., 2007). On the other hand, *Q. petraea* and *Q. rubra* indicated lower tannin content compared to the findings of the present study (33.9 and 29.7 mg pyrogallol g^{-1}) (Luczaj *et al*., 2014). This discrepancy may arise from varying techniques for drying, extracting, and analyzing acorn samples. According to this point, it has been demonstrated that the tannin level in *Q. robur* acorns ranged from 7.8% to 20.4%, depending on the specific techniques utilized for drying and extracting the samples (Rakić *et al*., 2007). Furthermore, certain specific procedures have an impact on the levels and composition of tannin. For example, the tannin content is enhanced through extract drying, whereas thermal treatment diminishes the concentration of tannin in comparison to the initial sample. This suggests that the processing methods used on extracts revealed a significant influence on the final composition of tannins (Rakić *et al*., 2007; Luczaj *et al*., 2014).

Quantity of gallic acid

Gallic acid was identified in the extract of kernel and pericarp from *Q. castaneifolia* and *Q. brantii* using HPLC (Table 2). The content of gallic acid was much higher in *Q. castaneifolia* samples (15.5 and 11.6 mg g^{-1} dry extract in kernel and pericarp, respectively) than those in *Q. brantii* (10.3 and 5.3 mg g^{-1} dry extract in kernel and pericarp, respectively). According to Rakić *et al*. (2007), the amount of gallic acid found in *Q. robur* and *Q. cerris* was determined to be 0.5% and 0.3%. An additional study employing the HPTLC method determined that *Q. griffithii* contained 1.9% gallic acid (Tsering et al., 2014). According to these results, the analysis method and mobile phase composition play a crucial role in accurately determining the amount of gallic acid. Interestingly, the aqueous extract of *Q. infectoria* gall contains a higher concentration of gallic acid compared to the methanolic and ethanolic extracts which underscored the importance of the extraction solvent in determining the quantity of gallic acid obtained (Syukriah *et al*., 2014).

Essential oil composition

The essential oil composition was analyzed by GC-MS and reported in Table 3. The main constituents of *Q. brantii* were (*2E,4Z*)-decadienal (16.0%), (*2E,4E*)-decadienal (10.6%), and *n*-nonanal (10.2%), while *Q. castaneifolia* essential oil consisted mainly of (*2E,4Z*)-decadienal (21.2%), limonene (19.0%), hexanal (16.4%), and (*2E,4E*)-decadienal (10.1%). *Q. castaneifolia* acorn oil was characterized by a high percentage of monoterpene hydrocarbons (20.9%) and a low

contribution of oxygenated monoterpenes (0.4%). On the other hand, *Q. brantii* contained equal amounts of monoterpene hydrocarbons and oxygenated monoterpenes. Limonene was the main monoterpene found in both *Q. castaneifolia* and *Q. brantii*, with relative amounts of 19% and 4.1%, respectively. The results indicated that the essential oils of these *Quercus* species displayed distinct compositions. Although there are certain similarities in the compounds found in both species, there are notable disparities in the quantity and quality of the samples. These discrepancies in the essential oil composition can be attributed to a range of factors, such as the season of harvest, the method of extraction, and the geographical origin.

Table 3. Essential oil composition (%) of the acorn kernel of *Quercus castaneifolia* and *Q. brantii.*

Components [a]	***RT*** [b]	***Q. castaneifolia* (%)**	***Q. brantii* (%)**	***KI*** [c]
Octane	5.6	-	1	799
Hexanal	5.9	16.4	8.3	807
2-Octene	6.1	-	0.2	812
Heptanal	10.2	1.1	0.8	909
α-Pinene	11.4	0.6	-	934
(*E*)-2-Heptenal	13.1	-	0.4	966
Benzaldehyde	13.6	-	0.5	976
1-Octen-3-ol	14.1	-	0.5	986
β-Myrcene	14.4	0.6	0.2	992
2-Pentyl furan	14.5	-	1	994
Furfuryl methyl sulfide	14.5	0.5	-	994
1,2,4-Trimethylbenzene	14.8	-	1.7	1000
1,3,5-Trimethylbenzene	14.8	1	-	1000
(*2E,4E*)-Heptadienal	15.2	1.4	0.6	1008
n-Octanal	15.3	-	1.4	1011
Mesitylene	16.2	1	-	1028
o-Cymene	16.3	0.8	0.9	1030
Limonene	16.5	19	4.1	1033
1,8-Cineole	16.7	0.4	-	1037
Indane	17.0	-	0.3	1042
1-Methyl-3-propyl benzene	17.7	-	0.6	1056
Phenyl propanal	17.7	0.7	-	1056
Benzene acetaldehyde	17.8	-	0.8	1058
2-Ethyl-1,4-dimethyl benzene	18.0	-	1.5	1063
1-Methyl-3-1-methylethyl Benzene	18.0	1.5	-	1063
(*E*)-2-Octenal	18.3	-	0.8	1068
(*2E*)-Octen-1-al	18.3	2.1	0.3	1069
4-Ethyl-1,2-dimethyl benzene	18.3	2.1	-	1081
1-methyl-2-propyl benzene	18.4	-	0.3	1070
n-Octanol	18.8	-	0.4	1078
p-Cymenene	19.0	-	1	1081

Components [a]	***RT*** [b]	***Q. castaneifolia* (%)**	***Q. brantii* (%)**	***KI*** [c]
2-Ethyl-1,4-dimethyl benzene	19.0	1	-	1090
n-Nonanal	19.5	1	10.2	1113
n-Undecane	19.9	-	0.3	1099
1,2,3,5-Tetramethyl benzene	20.6	2.4	-	1123
4-Ethyl-1,2-dimethyl benzene	21.1	0.6	-	1081
1,2,3,4-Tetramethyl benzene	21.3	0.8	0.8	1127
1,2,4,5-Tetramethyl benzene	22.9	-	0.9	1158
(*E*)-2-Nonenal	23.5	-	1.2	1170
Naphthalene	24.9	-	1.2	1199
n-Decanal	25.6	-	0.6	1214
Pulegone	27.3	-	0.7	1251
(*2E*)-Decenal	28.4	-	1.6	1272
1,2,3,4-Tetrahydro-6-methyl naphthalene	28.5	-	0.6	1276
Nonanoic acid	28.8	-	1.6	1282
n-Tridecane	29.6	-	1.1	1299
(*2E,4E*)-Decadienal	29.9	10.1	10.6	1306
2-Methyl naphthalene	30.1	2.7	2.1	1311
Carvacrol	30.2	-	2.7	1313
1-Methyl naphthalene	30.8	1.3	2	1327
(*2E,4Z*)-Decadienal	31.1	21.2	16	1332
2-Undecenal	33.0	-	1.6	1375
n-Tetradecane	34.0	-	0.9	1399
1-Ethyl naphthalene	34.5	-	0.4	1409
2,6-Dimethyl naphthalene	34.9	0.3	0.4	1421
2,7-Dimethyl naphthalene	35.0	-	0.4	1423
Naphthalene, 2,3-dimethyl	35.6	-	0.8	1435
1,6-Dimethyl naphthalene	35.7	-	0.5	1440
1,7-Dimethyl naphthalene	36.5	-	0.6	1456
n-Pentadecane	38.2	-	0.3	1499
2,5-Bis(1,1-dimethylethyl) phenol	38.9	0.7	3.4	1516
n-Hexadecane	42.2	-	0.4	1599
Benzophenone	44.2	-	0.3	1650
n-Heptadecane	45.9	-	0.4	1699
n-Octadecane	49.5	-	0.3	1799
Methyl hexadecanoate	53.9	0.6	0.4	1931
Hexadecanoic acid	55.4	-	1.5	1974
Ethyl hexadecanoate	56.1	-	0.8	1997
Total Identified		**91.9**	**95.2**	

[a] The arrangement of the compounds was organized based on their retention time on an HP-5MS capillary column; [b] Retention times (min); [c] Kovats Index was determined by GC-FID on an HP-5MS column.

Antioxidant activity

Antioxidant activity of the extracts obtained from the kernel and pericarp of *Q. castaneifolia and Q. brantii* were evaluated by DPPH and ABTS-radical scavenging assays. According to Table 4, all extracts displayed remarkable scavenging activities on DPPH and ABTS. This is attributed to the presence of phenolic compounds, which act as important antioxidants due to their ability to donate hydrogen atoms or electrons to form stable radical intermediates. The minimum concentration to 50% reduction of DPPH and ABTS radicals were approximately 6.6 µg mL^{-1} and 61.3 µg mL^{-1} in *Q. castaneifolia* kernel and *Q. brantii* pericarp, respectively. The ABTS radical scavenging activity showed a correlation with the phenolic content of the extracts, despite the results differed from those obtained through DPPH radical scavenging. This disparity could potentially be attributed to the diverse chemical compositions of the extracts and the dissimilar ways in which phenolics and free radicals interact in the two methods. Furthermore, the ABTS assay is frequently recommended as a superior alternative to the DPPH method, as it provides a more accurate assessment of the antioxidant potential of distinct substances and works well for both hydrophilic and lipophilic compounds, likewise heavily pigmented specimens resembling the acorn extract.

Table 4. Half-maximal inhibitory concentration (IC_{50}) values for DPPH and ABTS-radical scavenging activity by the acorn kernel and pericarp of *Quercus castaneifolia* and *Q. brantii*. Results were presented as mean ± standard deviation (SD) based on three repeated experiments.

Antioxidant assay	**IC_{50} values (µg mL^{-1}) for radical scavenging activity**			
	Q. castaneifolia		***Q. brantii***	
	kernel	**pericarp**	**kernel**	**pericarp**
DPPH	6.6 ± 0.2	7.6 ± 0.5	9.8 ± 0.2	13.0 ± 1.4
ABTS	129.3 ± 3.2	115.2 ± 6.6	148.5 ± 2.0	61.3 ± 1.4

Antibacterial activity

Based on the traditional use of acorns in medicine and applied topically to burns and wounds to prevent infection, the antibacterial activity of the acorn kernel and pericarp extracts from *Q. castaneifolia* and *Q. brantii* was evaluated against some gram-positive (*S. aureus*, *S. epidermidis*) and gram-negative (*P. aeruginosa*, and *E. coli*) bacteria (Table 5). The obtained results indicated that *Q. castaneifolia* pericarp and kernel extracts exhibited higher antibacterial activity compared to *Q. brantii* suggesting that *Q. castaneifolia* contains bioactive compounds with stronger antimicrobial potential than *Q. brantii*. The ethanolic extract of *Q. persica* demonstrated an effective antibacterial activity against *Bacillus subtilis*, *E. coli*, *Klebsiella pneumonia*, and *S. aureus* (Nourafcan *et al*., 2013), while *Q. floribunda* acorn extract exhibited a slight antibacterial effect on the same bacteria. Moreover, the extracts of *Q. ilex* and *Q. suber* demonstrated the ability to inhibit methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MSRA), while not affecting *E. coli* and *P. aeruginosa* (Silva *et al*., 2023). This aligns with a prior study

that indicated that aqueous acorn pericarp extracts inhibited MRSA and vancomycin-resistant *S. aureus* (VRSA) while exhibiting minimal against tested gram-negative strains (Sung *et al*., 2012). Interestingly, methanolic *Q. ilex* extract was found to inhibit the growth of *E. coli*, whereas the growth of *S. aureus* remains unaffected, thus contradicting the results of previous studies (Güllüce *et al*., 2004). These variations in antibacterial activity may be attributed to the type of solvent used for extraction, the time of harvesting, the specific species of oak tree, and methods employed for measurement. Furthermore, it may be associated with the phenolic content and quantity present in the acorns. Considering their antimicrobial properties, acorns find applications as preservatives in the food and cosmetic industries (Vinha *et al*., 2016).

Table 5. Half-maximal inhibitory concentration (IC_{50}) of the acorn kernel and pericarp of *Quercus castaneifolia* and *Q. brantii* using agar well-diffusion method.

Microorganism	**IC_{50} value (µg mL^{-1})**			
	Q. castaneifolia		***Q. brantii***	
	kernel	**pericarp**	**kernel**	**pericarp**
Staphylococcus aureus	12.5	12.5	25	25
Staphylococcus epidermidis	12.5	12.5	25	25
Escherichia coli	6.25	6.25	12.5	12.5
Pseudomonas aeruginosa	12.5	12.5	25	25

Conclusions

In this study, the phytochemical profiles and biological activities of the kernel and pericarp of acorns were evaluated in two *Quercus* species, *Q. castaneifolia* and *Q. brantii*. The results show that acorns are rich in phytoconstituents, particularly polyphenols, which possess significant antioxidant and antimicrobial properties, making them valuable for functional foods and nutraceuticals. Additionally, their high content of essential fatty acids, such as oleic acid and linoleic acid, underscores their potential as a nutritious food source. Nevertheless, further research is needed to evaluate the safety, toxicity, and detailed composition of acorns, to better understand their potential applications in food products.

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