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Research Article

Phytochemical characterization and *in vitro* antioxidant properties of endemic *Quercus vulcanica* bark

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Abstract: The bark of the endemic Kasnak oak (*Quercus vulcanica*) was analyzed to determine its phytochemical composition, including total phenolic, flavonoid, and condensed tannin contents, as well as its antiradical and reducing antioxidant properties. The results showed a high yield of hydroethanolic extract (17.4%), with a moderate total phenolic content of 420 mg GAE/g extract. Flavonoid and condensed tannin contents were substantial, reaching 179 mg/g extract and 90 mg/g extract, respectively. *In vitro* antioxidant assays demonstrated strong activity, with notably high DPPH values of 635 mg TE/g extract, while Fe (III)-reducing capacity was moderate to high (2.6 mM/g extract). *Q. vulcanica* bark differed from that of other oak species in its higher extract content and elevated FRAP antioxidant activity. Principal component analysis revealed positive correlations among flavonoid, total phenolic, and condensed tannin contents. These findings demonstrated *Q. vulcanica* bark as a promising source of bioactive compounds.

INTRODUCTION

Tree bark is a largely underutilized form of biomass. Compared to wood and energy crops, it generally contains lower levels of polysaccharides and higher concentrations of extractives and inorganic matter (Fengel & Wegener, 1984). The reduced polysaccharide content, which includes cellulose and hemicelluloses, results in a lower sugar yield after hydrolysis, making bark less suitable for applications such as pulp and paper or bioethanol production (Sjostrom, 1993). Moreover, the elevated levels of extractives and inorganics can cause operational issues during thermal conversion, including tar formation, slagging, and fouling (Gusta *et al.*, 2009; Niu *et al.*, 2016). Despite these drawbacks, such characteristics also offer potential advantages in extractive-based biorefineries. In these processes, biomass is fractionated to recover valuable extracts and materials before being converted into solid fuel or biochar.

Biomass extractives are compounds soluble in organic solvents and can be broadly grouped into polar and non-polar compounds (Fengel & Wegener, 1984). They are further classified based on their chemical nature into aliphatic compounds (e.g., fats and waxes), terpenes and terpenoids (e.g., resin acids and friedelin), and phenolic compounds (Fengel & Wegener, 1984, Sjostrom, 1993). Phenolic substances constitute a diverse and important class, encompassing

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hydrolyzable tannins, flavonoids, lignans, stilbene derivatives, and tropolones (Fengel & Wegener, 1984).

Phenolic molecules are known for their potent antioxidant activity. Consequently, they are frequently investigated as scavenging or reducing agents in the development of nutraceutical, pharmaceutical, and cosmetic products. Despite their promising properties, the use of natural antioxidants remains limited, with most bioactive compounds still being synthesized chemically. However, growing concerns about the potential health risks associated with synthetic compounds have increased interest in natural alternatives (Rumpf *et al*., 2023). Unlike synthetic agents, natural extracts consist of a diverse array of bioactive constituents. For instance, flavonoids encompass various subclasses, including flavanols, flavones, isoflavones, flavanones, anthocyanins, and flavan-3-ols. Given this complexity, it is essential to classify and quantify these compound groups and evaluate their bioactive potential.

A total of 23 oak species naturally grow in Türkiye with 4 of them being endemic (Baran *et al*., 2019). Oak biomass usually contains a large group of phenolics and exhibits bioactivity (Rosales-Castro *et al*., 2012; Popovic *et al*. 2013; Baran *et al*., 2019; Söhretoğlu & Renda, 2020). Among these, the kasnak oak (*Quercus vulcanica* Boiss. ex Kotschy) is an endemic species native to Turkey. The chemical composition of *Q. vulcanica* bark indicates a substantial extract content, comprising approximately 20% of the biomass dry weight, with phenolic compounds making up around 90% of the total extracts (Şen *et al*. 2024). The high water and alkali solubility of *Q. vulcanica* bark (Balaban & Uçar, 2001; Şen *et al*., 2024) suggest that the phenolic extractives from the bark could be valorized in a biorefinery process before the biomass is used for energy or fuel production. The biorefinery study of the *Q. vulcanica* bark revealed a particularly promising antioxidant activity determined by the thiobarbituric acid reactive substances (TBARS) method (Şen *et al*., 2024). However, further antioxidant and bioactivity tests are required to fully understand the bioactivity of *Q. vulcanica* bark. The phenolic composition of the *Q. vulcanica* bark was reported to contain condensed tannins and other phenolics (Balaban & Uçar, 2001). Since the phenolic profile of the biomass extracts plays an important role in bioactivity, the quantification of total phenols, flavonoids, and condensed tannins is necessary.

The present study aims to investigate the phenolic composition and antioxidant activity of *Q. vulcanica* bark by determining the hydroethanolic extract yield, total phenolic content, flavonoid and condensed tannin contents, as well as *in vitro* antiradical and reducing antioxidant properties. The findings contribute to ongoing research focused on screening and producing potential bioactive compounds from tree barks, with a particular emphasis on oak barks.

MATERIALS and METHODS

Materials

Quercus vulcanica bark samples were collected by randomly sampling five healthy and mature (age greater than 40) trees at breast height (1.5 m) in Isparta, Turkey in 2024. Approximately 250 g of bark was air-dried at ambient temperature (20 ± 1 °C) for five days, then milled and sieved to obtain particles in the 40–60 mesh range (250–420 μm). The bark particles were stored in air-sealed bags until the time of the following analyses.

Extraction

The extraction of polar compounds involved the determination of total phenolic content (TPC), flavonoids, and condensed tannins. Approximately 1 g of the 40–60 mesh fraction of *Q. vulcanica* bark was extracted using a 50:50 (v/v) ethanol–water solution at a solid–liquid ratio of 1:10 (m/v) for 60 minutes at controlled temperature conditions (50 °C ± 2 °C) in an ultrasonic bath. Insoluble materials were removed by filtration, and the resulting supernatant was stored at 4 °C until further analysis of phenolic composition and antioxidant activity (Şen *et al*. 2023). All subsequent phenolic composition and antioxidant tests were performed with a minimum of four replicates.

Total Phenolic Content

The Folin–Ciocalteu colorimetric assay was employed to measure the total phenolic content of *Q. vulcanica* bark, and the results were reported as milligrams of gallic acid equivalents (GAE) per gram of dry extract (Singleton *et al*. 1999).

Flavonoids Content

Flavonoid content in *Q. vulcanica* bark was quantified using the aluminum chloride ($AlCl_3$) colorimetric assay, and the results were reported as milligrams of (+)-catechin equivalents (CE) per gram of dry extract (Ferreira *et al*. 2018).

Condensed Tannins Content

The condensed tannin content in *Q. vulcanica* bark was assessed using the vanillin-H_2SO_4 method, with the results expressed as milligrams of (+)-catechin equivalents (CE) per gram of dry extract (Abdalla *et al*. 2014; Broadhurst & Jones, 1978;).

DPPH Antioxidant Assay

The *in vitro* antioxidant activity of *Q. vulcanica* bark extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) assay. The DPPH antioxidant activity was expressed as the concentration of extract needed to reduce DPPH by 50% (IC_{50}) and as Trolox equivalent antioxidant capacity (TE) on a dry extract basis (mg Trolox/g dry extract) (Sharma & Bhat, 2009).

FRAP Antioxidant Assay

The ferric reducing antioxidant power (FRAP) assay was performed following the method of Benzie and Strain. Aqueous solutions of known Fe (II) concentrations, ranging from 0 to 1500 µmol/L ($FeSO_4.7H_2O$), were used to create the calibration curve. The results were expressed as millimoles of Fe (II) per gram of extract, with Trolox and catechin used as reference compounds (Benzie & Strain, 1996).

Statistical Analysis

Microsoft Excel ® and Matlab ® were used to perform statistical analysis. The statistical analysis involved determining the coefficient of variation (%), constructing a correlation matrix, and conducting principal component analysis to evaluate the relationships between the phenolic composition properties of *Q. vulcanica* bark. These properties included extract yield (EY), total phenolic content (TPC), flavonoid content (FC), condensed tannin content (CT), DPPH antioxidant activity, and FRAP antioxidant activity.

RESULTS and DISCUSSION

Phenolic Composition

The results of the phenolic composition test indicate that the hydroethanolic extract content of *Q. vulcanica* bark is approximately 17%. The phenolic composition of *Q. vulcanica* bark is highly variable and composed mainly of flavonoids and condensed tannins with 179 mg CE g^{-1} and 90 CE g^{-1} extract, respectively. The DPPH antioxidant activity of the bark is high (635 mg TE g^{-1} extract), while the FRAP antioxidant activity is moderate (2.6 mM g^{-1} extract).

The phenolic composition assays demonstrated high precision, with coefficient of variation values less than 10% except for condensed tannins content, which also exhibited an acceptable coefficient of variation (Table 1).

The high extract content of bark agrees with previous result (Şen *et al*., 2024). In fact, the extract content of the bark is among the highest values reported for similar biomass types and the highest among the oak barks previously reported (Table 2). The phenolic content of bark, on the other hand, appears to be moderate as in the case of Eucalyptus biomass (Miranda *et al*. 2016) (Table 1).

Table 1. Coefficient of variation values (%) between phenolic properties *Quercus vulcanica* bark (EY: extract yield; TPC: total phenolic content; FC: flavonoid content; CT: condensed tannin content; DPPH: antioxidant content; FRAP: antioxidant content)

Test	CV (%)
EY	6.1
TPC	3.4
FC	5.9
CT	13.4
DPPH	5.3
FRAP	6.0

Table 2. Extract yield and total phenolic content of *Quercus vulcanica* bark.

Bark	Extract yield (%)	TPC (mg GAE g^{-1} extract)	Reference
Quercus vulcanica	17.4 ± 1.4	419.7 ± 14.3	The present study
Quercus faginea	6.4 ± 1.6	630.3 ± 127.8	Ferreira *et al*. 2018
*Quercus rotundifolia *(E)*	6.4 ± 2.8	572.8 ± 67.8	Sousa *et al*. 2021
*Quercus rotundifolia **(W)*	9.3 ± 1.3	219.5 ± 33.1	Sousa *et al*. 2021
Quercus suber cork	10.1 ± (ns)	786.9 ± 55.5	Touati *et al*. 2015
Quercus cerris cork	6.9 ± 3.7	733.8 ± 35.5	Şen *et al*. 2020
Quercus cerris phloem	4.9 ± 1.7	255.2 ± 8.2	Şen *et al*. 2020
Eucalyptus sideroxylon	50.0 ± 0.5	440.7 ± 35.2	Miranda *et al*. 2016
Eucalyptus urophylla hybrids	9.5 ± 3.3	380.9 ± 28.8	Sartori *et al*. 2018
Copaifera langsdorffii	12.8	589.2	Carmo *et al*. 2016

ns: not specified *Ethanol extract, **Water extract

The flavonoid content of *Q. vulcanica* bark is similar to other biomass types such as *Eucalyptus urophylla*, *Quercus cerris* phloem, and *Q. rotundifolia*, while its condensed tannin content was higher than those biomasses, suggesting that polymeric flavonoids make up a significant contribution to total flavonoid content in *Q. vulcanica* bark (Table 3).

Table 3. Phenolic composition of *Quercus vulcanica* bark.

Bark	Flavonoids (mg g^{-1} extract)	Condensed tannins (mg g^{-1} extract)	Reference
Quercus vulcanica	179.0 ± 10.5	90.1 ± 12.1	The present study
Quercus faginea	204.7 ± 32.6	220.7 ± 54.2	Ferreira *et al*. 2018
*Quercus rotundifolia (E)**	247.6 ± 49.1	249.1 ± 112.5	Sousa *et al*. 2021
*Quercus rotundifolia (W)***	162.5 ± 45.5	41.2 ± 9.8	Sousa *et al*. 2021
Quercus suber cork	49.0 ± 2.0	29.5 ± 4.0	Touati *et al*. 2015
Quercus cerris cork	306.6 ± 16.4	419.4 ± 68.5	Şen *et al*. 2020
Quercus cerris phloem	190.0 ± 4.6	131.4 ± 11.4	Şen *et al*. 2020
Eucalyptus sideroxylon	204.4 ± 26.4	395.0 ± 51.9	Miranda *et al*. 2016
Eucalyptus urophylla hybrids	178.4 ± 36.2	67.7 ± 9.3	Sartori *et al*. 2018
Copaifera langsdorffii	441.9	54.8	Carmo *et al*. 2016

*E: Ethanol extract, **W: Water extract

In vitro Antioxidant Properties

The antioxidant activity of *Q. vulcanica* bark was assessed by two different antioxidant assays. The DPPH method indicated a high antioxidant activity that is comparable to other oak barks, while the FRAP method showed moderate to high antioxidant activity (Table 4). The difference between the two methods suggests that antioxidant activity of the extracts mainly occurs by hydrogen atom transfer, and single electron transfer rather than solely by single electron transfer and the extracts may be used as radical-scavenging agents. The overall antioxidant test results agree with previous TBARS antioxidant activity results, further confirming the antioxidant activity of *Q. vulcanica* bark extracts (Şen *et al*. 2024).

Table 4. *In vitro* antioxidant properties of *Quercus vulcanica* bark.

Bark	DPPH (mg TE g^{-1} extract)	IC_{50} (µg mL^{-1})	FRAP (mM g^{-1} extract)	Reference
Quercus vulcanica	634.9 ± 33.7	4.6 ± 0.2	2.6 ± 0.2	The present study
Q. faginea	1576.1 ± 444.9	2.6±0.5	4.4 ± 0.8	Ferreira *et al*. 2018
Quercus rotundifolia (E)	1043.8±306.4	4.4±1.1	4.3 ± 2.1	Sousa *et al*. 2021
Quercus rotundifolia (W)	940.2±257.9	4.7±1.4	1.3 ± 0.4	Sousa *et al*. 2021
Quercus suber cork		5.7±0.0		Touati *et al*. 2015
Quercus cerris cork	729.5±13.3	4.6± 0.1	3.3 ± 0.3	Şen *et al*. 2020
Quercus cerris phloem	435.8±14.0	1.8± 0.1	1.8 ± 0.1	Şen *et al*. 2020
Eucalyptus sideroxylon	648.8 ± 4.8	2.3 ± 0.8	5.2 ± 0.4	Miranda *et al*. 2016
Eucalyptus urophylla hybrids	585.5 ± 105.2	5.4 ± 0.7		Sartori *et al*. 2018
Copaifera langsdorffii	720.3	3.9		Carmo *et al*. 2016

Correlations Between Phenolic Properties and Antioxidant Activity

After performing the phenolic composition and antioxidant tests, the correlations between different tests were analyzed by using a pairwise Pearson correlation matrix (Table 5) followed by a principal component analysis (PCA). The Pearson correlation matrix indicated that extract yield is strongly correlated with antioxidant properties, flavonoid content, and condensed tannin content. The total phenolic content is strongly correlated with flavonoid content, while flavonoid content is positively correlated with antioxidant content. Condensed tannin content also showed a strong correlation with DPPH antioxidant content.

Table 5. Pairwise correlation matrix between phenolic properties *Quercus vulcanica* bark.

Test	Extract yield	TPC	FC	CT	DPPH	FRAP
Extract yield	1					
TPC	0.538321	1				
FC	0.860985	0.842576	1			
CT	0.857326	0.035265	0.487897	1		
DPPH	0.953547	0.305391	0.75104	0.925743	1	
FRAP	0.869794	0.686333	0.765569	0.655015	0.692923	1

While the Pearson correlation matrix highlighted strong linear associations between phenolic variables and antioxidant activities, the PCA results indicate different multivariate interactions, possibly due to uncaptured correlations by pairwise correlations alone.

The results of principal component analysis are shown in Figure 1 and Figure 2. When the phenolic extraction properties were analyzed with DPPH antioxidant activity, 2 principal components were obtained, explaining approximately 84% and 15% of the variance (Figure 1). On the other hand, when the phenolic extraction properties are analyzed with FRAP antioxidant activity, 2 principal components were obtained, explaining approximately 66% and 33% of the variance.

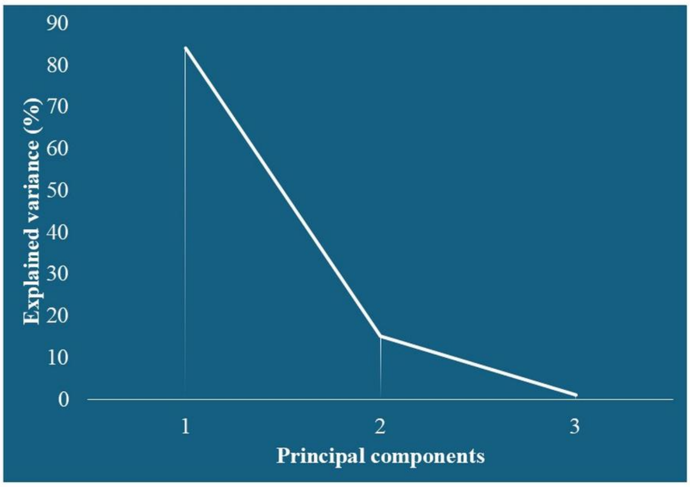

Figure 1. The explained variances between extract yield, phenolic composition and DPPH antioxidant activity.

In general, flavonoid content showed positive correlations with total phenolic content or condensed tannin content (Figure 2). The antioxidant contents correlated weakly with total phenolic, flavonoid, and condensed tannin contents, while extract yield did not show a significant contribution to the principal components. These results suggest that the phenolic composition of *Q. vulcanica* bark is predominantly formed by soluble conjugates (Anokwuru *et al*. 2024).

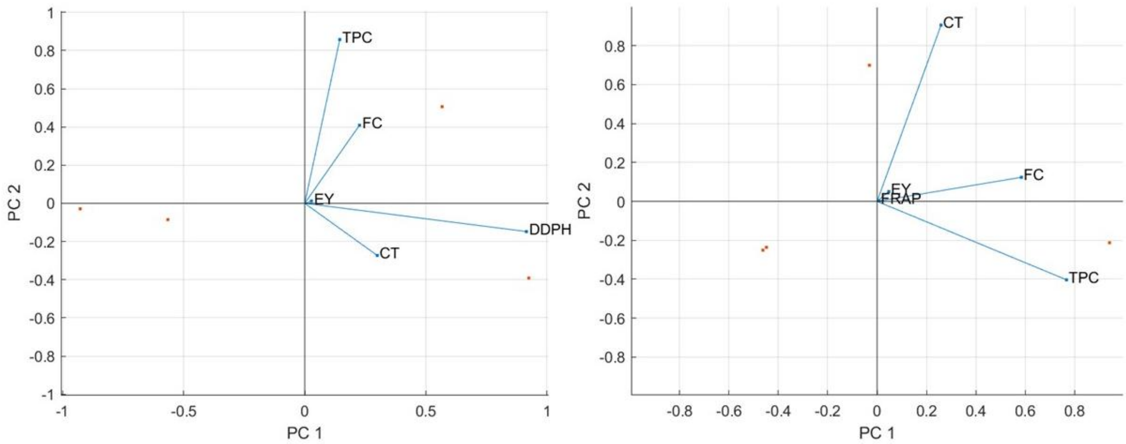

Figure 2. Biplots showing correlations between phenolic properties and DPPH antioxidant activity (on the left) and FRAP antioxidant activity (on the right).

The phytochemical analysis and notable antioxidant potential of *Q. vulcanica* bark indicate that phenolic-rich extracts from this species provide sustainable alternatives to synthetic substances, including synthetic antioxidants for food preservation, food packaging, and cosmetic applications (Albuquerque *et al*. 2021). Furthermore, the hydroethanolic extracts of the bark promote the conservation of the endemic species by demonstrating sustainable use possibilities for local communities. As a result, the use of bark extracts contributes to the achievement of the United Nations' sustainable development goals.

DISCUSSION and CONCLUSION

The extract yield, phenolic composition, and antioxidant activities of *Quercus vulcanica* bark were investigated. The results indicated that *Q. vulcanica* bark is a rich source of phenolic compounds with significant antioxidant activity. The radical scavenging activity, as determined by DPPH, was notably high, with a value exceeding 635 mg TE/g extract, while the Fe (III) reducing activity was moderate to high (2.6 mM g^{-1} extract). Principal component analysis revealed positive correlations between flavonoid, total phenolic, and condensed tannin contents. The overall results indicate that *Q. vulcanica* bark is a promising biomaterial for the extraction of bioactive compounds, particularly antioxidant compounds for the food preservation, food packaging, and cosmetic industries.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

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