

RESEARCH PAPER

Effects of the supercritical fluid enhanced with co-solvent on the phenolic and flavonoid contents of *Pistacia terebinthus* extracts

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Abstract

The turpentine tree (*Pistacia terebinthus* L.) contains valuable bioactive compounds such as phenolic acids, flavonoids, and terpenes, attracting scientific interest beyond its culinary and aromatic uses. This study evaluated the effects of supercritical CO₂ (SCCO₂) extraction, with and without ethanol (EtOH) as a co-solvent, on the phenolic, flavonoid, anthocyanin, and antioxidant contents of *P. terebinthus* extracts. The addition of EtOH significantly reduced total phenolic content (32.10 ± 3.80 mg GA/100 g DM) compared to SCCO₂ alone (43.59 ± 4.60 mg GA/100 g DM; *P* < 0.043), possibly due to ethanol interference, compound-solvent interactions, or matrix-related factors. Flavonoid contents showed similar trends, with pure SCCO₂ yielding the highest levels. This result may be attributed to the hydrophobic nature and complex polyphenolic structures of the flavonoids in the extracts. Both SCCO₂ and SCCO₂-EtOH methods outperformed ultrasound-assisted (US) extraction in flavonoid yield (*P* < 0.001). However, US extraction resulted in the highest anthocyanin content (5.01 ± 0.49 mg TAC/100 g DM), likely due to its low-temperature conditions that prevent thermal degradation. Overall, SCCO₂ extraction with EtOH co-solvent appears to be a promising approach for efficiently extracting flavonoids, anthocyanins, and antioxidant compounds from *P. terebinthus*.

Introduction

Phenolic chemicals represent a group of plant-derived secondary metabolites characterized by one or several hydroxyl groups bonded to aromatic ring structures (Leyva-Jiménez et al., 2020). These compounds have gained interest for extraction and product application across food, pharmaceuticals, and cosmetics because of their antimicrobial, anti-inflammatory, and antioxidant biological properties (Leyva-Jiménez et al., 2020; Tyśkiewicz et al., 2018; Woźniak et al., 2017). The structural diversity of phenolic compounds and other bioactive plant constituents like flavonoids presents extraction challenges because researchers must balance both

polarity and solubility considerations (Leyva-Jiménez et al., 2020).

The use of supercritical fluid extraction (SFE), which operates on fluids in their phase above the critical point, has demonstrated potential as a successful method for selectively isolating phenolic and flavonoid compounds from natural sources (Tyśkiewicz et al., 2018). Adding a co-solvent in supercritical fluid extraction improves solvation and mass transfer properties, which results in higher extraction yields and precise targeting of bioactive compounds (Abbasi et al., 2008; Chaitanya et al., 2015; Jiao & Kermanshahi pour, 2018; Leyva-Jiménez et al., 2020).

SFE has emerged as a powerful and environmentally sustainable method for isolating bioactive compounds, particularly from thermolabile plant materials. Utilizing fluids above their critical temperature and pressure, most commonly carbon dioxide, SFE offers several advantages including, low extraction temperatures, tunable solvating power, and solvent-free residue. These properties make it particularly suitable for the recovery of volatile and semi-volatile compounds while preserving their bioactivity ([Hernández-Alonso et al., 2016](#); [Tyśkiewicz et al., 2018](#)). The diffusivity and density of supercritical fluids allow efficient penetration into plant matrices and facilitate rapid mass transfer, resulting in high-purity extracts with minimal degradation. SFE has been widely applied for the extraction of phenolics, essential oils, and flavonoids from a variety of botanical sources ([Villalva et al., 2021](#)). Ultrasound-assisted extraction (US extraction), another advanced extraction approach, operates based on the phenomenon of acoustic cavitation, wherein high-frequency ultrasonic waves generate microbubbles that collapse violently, disrupting plant tissue and enhancing solvent penetration. This mechanical disruption of cell walls leads to the release of intracellular compounds, significantly increasing extraction yield and reducing both time and solvent consumption. US extraction is particularly valued for its low operational temperatures, which help retain the structural integrity and biological activity of phenolic compounds ([Chemat et al., 2017](#)). Moreover, US extraction has been shown to improve the efficiency of mass transfer processes and is compatible with both polar and non-polar solvents, making it a versatile tool in phytochemical extraction. Studies have demonstrated that US extraction outperforms conventional techniques in terms of phenolic yield and antioxidant potency in various plant species ([Machado-Carvalho et al., 2023](#); [Talmaciu et al., 2015](#)). Both SFE and US extraction represent key components of modern green extraction technologies, offering efficient, selective, and sustainable alternatives to traditional solvent-based methods. Their operational advantages, including enhanced mass transfer, reduced energy consumption, and protection of bioactive compounds, make them particularly suitable for the recovery of phenolics from natural matrices.

Among the many plant sources rich in phenolic compounds, members of the Anacardiaceae family, particularly the genus *Pistacia*, have attracted attention due to their traditional use and phytochemical richness. Since ancient times, the pistachio has been one of the most cultivated nuts in the Mediterranean area and has been consumed for several thousand of years. Historical accounts reveal that pistachios were widely cultivated in the Persian Empire, from which they gradually spread westward ([Hernández-Alonso et al., 2016](#)). The cultivation of pistachios extends across multiple regions, including the Middle East, the United States, and the Mediterranean area where they serve as both a

common snack and multiple culinary ingredients. The turpentine tree (*Pistacia terebinthus*) contains multiple bioactive compounds, including phenolic acids, flavonoids, and terpenes which scientific studies have associated with health advantages through antioxidant, anti-inflammatory, and antimicrobial properties ([Abbasi et al., 2008](#); [Leyva-Jiménez et al., 2020](#); [Tyśkiewicz et al., 2018](#)). Phenolic compounds represent a large and structurally diverse group of secondary metabolites characterized by one or more hydroxyl groups attached to aromatic rings ([Dai & Mumper, 2010](#)). They are broadly classified into several subgroups, including phenolic acids, flavonoids, tannins, lignans, and stilbenes. Among these, flavonoids are particularly abundant and important due to their diverse biological functions and structural variations. Flavonoids are themselves categorized into subclasses such as flavonols (e.g., quercetin), flavanols (e.g., catechin), flavones (e.g., apigenin), and anthocyanins (e.g., cyanidin), the latter of which contribute to pigmentation in plant tissues ([Panche et al., 2016](#)). *P. terebinthus*, also known as the terebinth tree, is a cousin to the pistachio tree (*Pistacia vera*) but is not generally used to produce a coffee-like beverage in Turkey in the same way that coffee beans are ([Gogus et al., 2011](#); [Yüksel et al., 2015](#)). Phytochemical studies have reported that fruits of *P. terebinthus* are rich in phenolic acids such as gallic acid and caffeic acid, and flavonoids like quercetin and catechin, which contribute to their strong antioxidant potential ([Özcan et al., 2020](#); [Uysal et al., 2022](#)). Additionally, anthocyanin derivatives have been identified, which contribute both to color and bioactivity ([Longo et al., 2007](#)). These compounds are primarily responsible for the antioxidant and anti-inflammatory properties attributed to the plant. It is worth mentioning that the term 'terebinth coffee' in Turkey usually means a drink made from the roasted fruits of *P. terebinthus* (menengiç), not the actual coffee beans. The specific and pleasant smell of roasted *P. terebinthus* fruits is caused by the thermal transformations that take place during the roasting process, similar to the process of roasting green coffee beans to obtain the distinct flavours of roasted coffee ([Gogus et al., 2011](#)). Furthermore, the essential oil composition of *P. terebinthus* fruits that were obtained from different regions of Turkey has been of particular interest to researchers, who have used advanced analytical techniques to define the chemical makeup of these aromatic plant products ([Özcan et al., 2009](#)). Apart from their culinary and fragrant use, the *P. terebinthus* plant and its products have also attracted much attention from scientists due to their pharmacological properties.

The main objective of this study was to investigate the effects of supercritical CO₂ extraction, enhanced by the addition of ethanol as a co-solvent, on the phenolic and flavonoid contents of extracts obtained from dried fruits of *Pistacia terebinthus*, a plant species native to the Mediterranean region. To provide a meaningful comparison, supercritical CO₂ extraction with or without

EtOH as a co-solvent was compared with ultrasonically assisted aqueous extraction, a widely used green extraction technique that relies on cavitation-induced cell disruption to increase compound recovery. The selection of US extraction with water as a comparative method was based on its operational simplicity, low-temperature processing, and proven efficacy in extracting thermolabile polyphenols from plant matrices. Moreover, since the use of EtOH as a co-solvent increases the polarity of the apolar CO₂ solvent, ultrasonic-assisted extraction with distilled water has been thought to serve as a benchmark technique, as it provides the most polar extraction medium. The effect of supercritical extraction parameters, particularly the presence of an ethanol co-solvent, on the total phenolic, flavonoid, and anthocyanin contents, as well as the antioxidant activities of the obtained extracts, was systematically evaluated by comparing them with those obtained by ultrasonically assisted aqueous extraction.

Materials and Method

Materials

Commercial liquid carbon dioxide (purity 99.9%) was purchased from a local supplier (Kırşehir, Turkey). Folin-Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), sodium carbonate (Na₂CO₃), aluminum chloride (AlCl₃), and potassium acetate (CH₃COOK) were supplied by Sigma. The standards used for the determination of flavonoids and phenolic acids, namely quercetin and gallic acid, were purchased from Sigma. Ethanol and methanol were of HPLC grade and purchased from Merck (Darmstadt, Germany). All reagents used were of analytical grade and were used without further purification.

Supercritical extraction of terebinthus fruits

Dried terebinthus fruits were purchased from the Elazığ local market and ground with a laboratory, type blender. Extraction experiments were conducted using a laboratory-scale SCCO₂ extraction system (Superex F-500, Pard Engineering and Automation Industry Trade Co. Ltd., Turkey), which consists of a 500 mL stainless steel extraction vessel equipped with a temperature control system and a high-pressure CO₂ pump (Figure 1). 240 g of terebinthus granules were weighed and loaded into a special bag of the supercritical extraction device for each extraction batch. The CO₂ flow rate was adjusted to 3 ± 0.3 ml/min. The extraction process was performed at a pressure of 300 bar and a temperature of 40 ± 5°C. Once the desired temperature and pressure were reached, the system was kept under static conditions for 20 min to allow for solubilization. Subsequently, CO₂ flow was initiated, and the dynamic extraction phase continued for 90 min. For co-solvent-assisted extractions (SCCO₂-EtOH), ethanol (2.0% wt) was pumped into the system as a co-solvent to enhance the solubility of polar compounds. The collected extracts were stored at +4°C until further analysis.



Figure 1. Supercritical CO₂ extraction device used to obtain the terebinthus fruit extracts.

Ultrasonic aqueous extraction was also performed to compare the polarity effect of non-polar supercritical CO₂ on the phytochemical content of terebinthus extracts with their aqueous counterparts. SCCO₂, SCCO₂-EtOH, and US extractions were carried out in triplicate and results obtained were expressed as mean ± standard deviation (SD).

Ultrasonic-assisted aqueous extraction of terebinthus

Ultrasound-assisted extraction (US extraction, Scientz, JY92-IIN, China) was performed with slight modifications to the procedure reported by Hefied et al. (2023) (Hefied et al., 2023) (Figure 2). For the extraction, 10 g of ground *P. terebinthus* fruit powder was mixed with 200 mL of distilled water (1:20 w/v) in a 500 mL glass bottle. The mixture was subjected to ultrasonic treatment using an ultrasonic processor set to 50% amplitude, with a pulse mode of 30 s on and 0.5 s off, at a constant temperature of 45 °C for a total duration of 40 min. The extraction temperature was monitored every five minutes throughout the process to ensure stability. Following sonication, the mixture was centrifuged at 6000 rpm for 10 min, and the supernatant was carefully collected. It was then filtered through a 0.45 µm membrane filter. To obtain the dry extract, the filtrate was freeze-dried at -50°C under a vacuum pressure of 0.07–0.1 mbar (Scientz-10N/C brand, Ningbo, China). The resulting powdered extract was stored at -20°C until further analysis.



Figure 2. Ultrasonic-assisted extraction device used to obtain the terebinthus fruit extracts.

Analytical protocols

Total phenol analysis

The total phenolic content in the extracts was determined using the Folin–Ciocalteu colorimetric method, following the protocol described previously (Yildiz-Ozturk et al., 2014, 2015). Briefly, 100 µL of each extract was mixed with 0.5 mL of Folin–Ciocalteu reagent and vortexed, followed by incubation at room temperature for 5 min. Then, 1.5 mL of saturated sodium carbonate solution (20%) was added, vortexed again, and the mixture was incubated for 1 h at room temperature in the dark. The absorbance was measured at 760 nm using a SP-3000 nano OPTIMA spectrophotometer. Total phenolic content was calculated based on a calibration curve prepared using gallic acid and expressed as mg gallic acid equivalent (GA) per gram of dry extract. All measurements were performed in duplicate for each sample taken at the end of the extractions.

Total flavonoid analysis

The principle of the method to be used is based on the reaction of flavonoids in the extracts with aluminum ion (Al^{3+}) to form a stable flavonoid- Al^{3+} complex. This complex forms a yellow color, and the intensity of the color is proportional to the flavonoid concentration. Flavonoid content is expressed as mg quercetin equivalent per 100 grams dry extract matter (mg Qe/100 g DM). 0.5 mL (in MeOH) was taken from the *P. terebinthus* extracts after 10-fold dilution and then mixed with MeOH (1.5 mL), aluminum chloride solution ($AlCl_3$, 0.1 ml, 10%), potassium acetate (CH_3COOK , 0.1 mL, 1 M) and distilled water (2.8 ml) and incubated at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm using an SP-3000 nano OPTIMA spectrophotometer (Yildiz-Ozturk et al., 2015). All measurements were performed in duplicate for each sample taken at the end of extractions.

Total anthocyanin analysis

The total amount of anthocyanin was analyzed by a pH differential method (Jeyaraj et al., 2022). This method is based on the fact that anthocyanins have different pigmentations at different pH values. In this way, the anthocyanin concentration in the samples can be measured spectrophotometrically thanks to the changes in coloration. The pH value of the extracts was adjusted to pH 1.00 using 0.025 M potassium chloride and to pH 4.5 using 0.4 M sodium acetate. HCl was used to adjust the solutions to the specified pH values. The absorbance of each extract was measured at 510 nm and 700 nm against a distilled water blank at pH 1.00 and pH 4.5 using a UV/visible spectrophotometer. All measurements were performed in duplicate for each sample taken at the end of extractions. Anthocyanin concentrations in the samples were calculated using the following equation:

$$\text{Anthocyanin pigment} \left(\frac{mg}{L} \right) = \frac{A \times MW \times DF \times 1000}{\epsilon \times l}$$

ΔA = Absorbance difference (difference in absorbance measured at pH 1.0 and pH 4.5 according to the applied method)

Absorbance difference is calculated according to the following equation:

$\Delta A = [(A_{510} - A_{700}) \text{ at pH 1.0}] - [(A_{510} - A_{700}) \text{ at pH 4.5}]$,

MW = molecular weight of cyanidin-3-glucoside (449.2 g/mol),

ϵ = extinction coefficient (26.900 L/mol.cm);

L = layer thickness of absorbance measuring cuvette (cm)

DF = dilution factor

Antioxidant activity analysis

This determination method is based on the measurement of the DPPH free radical scavenging effects of substances showing antioxidant activity on the stable and purple DPPH radical (Yildiz-Ozturk et al., 2015). The obtained *P. terebinthus* extracts were diluted 100-fold in 4 mL of MeOH and then 1 mM DPPH methanolic solution (0.5 ml) was added. The resulting mixture was stirred for 15 s and incubated in the dark for 30 min at room temperature. After incubation, the absorbance of the reaction mixture will be read against methanol at 517 nm using the SP-3000 nano OPTIMA spectrophotometer. All measurements were performed in duplicate for each sample taken at the end of extractions. The percentage of DPPH radical scavenging inhibition gives the sample concentration (IC_{50} value, µg/ml) required to inhibit 50% of DPPH free radical.

The absorbance values read were written in the given equation and the radical scavenging activity is calculated as a percentage.

$$\text{Inhibition\%} = \frac{A_{DPPH} - A_{Ext}}{A_{DPPH}} \times 100$$

A_{DPPH} : absorbance of the control solution (methanolic DPPH solution) without extract

A_{Ext} : absorbance of the solution containing the extract

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 9.0 software. One-way analysis of variance (one-way ANOVA) was applied to determine the differences between the groups, and Tukey's HSD (Honest Significant Difference) post hoc test was used for multiple comparisons after significance determination. The results obtained were expressed as mean \pm standard deviation (SD) and $P < 0.05$ was considered statistically significant.

Results and Discussion

Yields of Extractions

The results of extraction yields obtained with different methods are presented in Table 1. Extraction

yields depend on the method used and the polarity of the solvents, and the extraction yield is defined as the ratio of the mass of the extract obtained (M_{ext}) to the initial dry mass loaded into the device (M_0).

$$Y\% = \frac{M_{ext}}{M_0} \times 100$$

The US method provided higher extraction yields than other methods. It is well known that US extraction generally generates higher yields because of the destruction of the cellular wall. This is because of the enhanced mass transfer across the cell wall due to the collapse of the bubbles formed by cavitation (Monteiro et al., 2020). The different extraction yields obtained from supercritical fluid extraction or ultrasonic-assisted aqueous extraction in this study are in agreement with the literature (Argun et al., 2023; Confortin et al., 2019).

Table 1. The terebinth extract yields obtained using different methods

	SCCO ₂	SCCO ₂ -EtOH	US
Yield of extraction (%)	3.03 ± 0.38	4.10 ± 0.65	8.50 ± 1.07

Total Phenolic Contents (TPC) of Extracts

The phenolic content of different extracts was determined spectrophotometrically by Folin-Ciocalteu method, and the results are presented as gallic acid equivalents (GA) (Figure 3). Phenolic compounds such as flavonoids, phenolic acids and tannins have various biological activities, which are usually related to their antioxidant activity.

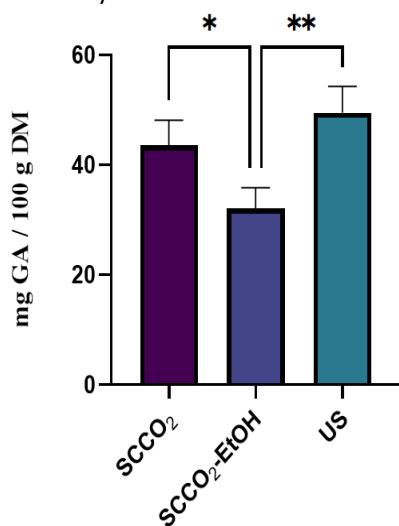


Figure 3. The total phenolic contents (mg gallic acid equivalent per 100 g dry matter) of terebinth fruit extracts obtained by different polarity extraction fluids. Data are represented as mean ± standard deviation. * $P < 0.05$; ** $P < 0.01$.

The total phenolic contents of the extracts obtained by SCCO₂, SCCO₂-EtOH, and US extraction were found to be 43.59 ± 4.60, 32.10 ± 3.80, and 49.51 ± 4.80 mg GA/100 g DM, respectively. When all methods were compared, it was found that the use of co-solvent resulted in significantly ($P < 0.043$) fewer phenolic compounds than the SCCO₂ method, and similarly, a

significantly ($P < 0.007$) lower content compared to the US method. The highest total phenolic contents were found in samples subjected to sonication for 40 min. The results indicated that the bioactive compound content and antioxidant activity were affected by the sonication process. It is thought that the cavitation promoted the migration of antioxidants and phenolic compounds into the extracts. Furthermore, probably the strongest possibility is that the high polarity index of water (PI: 9.2) may cause the solvent to interact more with the hydroxyl groups of the phenolics in terebinth granules.

The hydroxyl group (-OH) attached to an aromatic ring in phenolic compounds results in moderate polarity. The aromatic ring of phenolic compounds shows nonpolar characteristics, while the hydroxyl group enables good interactions with polar solvents, thus affecting their solubility in different solvents. The polar nature of water, with a polarity index of 9.0, enables it to dissolve various simple phenolic acids, yet its effectiveness at dissolving complex phenolic compounds is limited (Sultana et al., 2009). On the other hand, the unexpected outcome showed that the phenolic compound content decreased statistically significantly when SCCO₂ operated with a co-solvent. The addition of ethanol to the SCCO₂-EtOH system enhances the solubility and extraction efficiency of phenolic compounds significantly better than pure SCCO₂ because it addresses the polarity difference between SCCO₂ and phenolic compounds. The statistically significant decrease in the phenolic compound content when a co-solvent was used with SCCO₂ was an unexpected result. Generally, the addition of ethanol in the SCCO₂-EtOH system greatly improves the solubility and extraction efficiency of phenolic compounds compared to pure SCCO₂, as it helps overcome the polarity mismatch between SCCO₂ and phenolic compounds (Woźniak et al., 2017).

The extraction of phenolics is also influenced by several other factors, such as interference from ethanol co-solvent, solvent-compound interactions, or matrix-dependent factors. Although the ethanol increases the polarity of the solvent, it may also compete for the extraction sites or interfere with the extraction process. High ethanol concentration in the solution may decrease phenolic solubility in CO₂ by modifying the interactions between CO₂, ethanol, and the matrix (Mechi et al., 2023; Thoo et al., 2013; Vatai et al., 2009). It's also possible that the phenolic compounds in the terebinth sample may have a higher proportion of non-polar phenolics, so ethanol may not be necessary for optimal extraction.

Total Flavonoid Content of Extracts

Flavonoids at the subclass level, including flavonols, isoflavones, and anthocyanidins show distinct biological properties with antioxidant activity being one of them. The antioxidant properties of flavonoids stem from their specific molecular structures. The antioxidant properties of flavonoids depend heavily on the

placement of hydroxyl groups throughout their structures together with other structural elements (Hassanpour & Doroudi, 2023). It was found that the use of co-solvent did not show a statistically significant difference in the total flavonoid contents of the extracts when compared to the SCCO₂ method ($P < 0.05$) (Figure 4). However, the flavonoid content obtained with the US extraction method (7.29 ± 0.68) was found to be significantly lower compared to both the SCCO₂ (64.36 ± 6.00) and SCCO₂-EtOH (56.70 ± 5.80) methods ($P < 0.001$). This finding indicates that the US method exhibits lower efficiency, particularly in the extraction of flavonoids, compared to the SCCO₂ and co-solvent combinations.

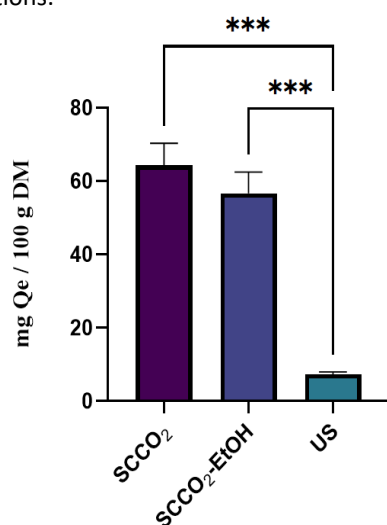


Figure 4. The total flavonoid contents (mg quercetin equivalent per 100 g dry matter) of terebinth fruit extracts obtained by different polarity extraction fluids. Data are represented as mean \pm standard deviation. *** $P < 0.001$.

The larger and more complex structure of flavonoids requires polar solvents for glycosides, but they can also be extracted with intermediate or non-polar solvents based on their hydrophilic/hydrophobic balance (Chávez-González et al., 2020; Hassanpour & Doroudi, 2023; Leyva-Jiménez et al., 2020). Typically, the use of SCCO₂ and ethanol as solvents creates a dual solvent system in which CO₂ functions as a non-polar solvent for lipophilic compounds and ethanol functions as a polar solvent to extract hydrophilic phenolic compounds such as flavonoids and phenolic acids (Galanakis et al., 2013). Previous studies have shown that the addition of a co-solvent like ethanol can significantly enhance the extraction yield and the isolation of specific groups of phenolics compared to using the supercritical fluid alone (Paes et al., 2014). However, similar to the phenolic contents of the extracts obtained with different extraction techniques in this study, the flavonoid contents also exhibited an unusual solubility and diffusion tendency. In this study, besides the fact that the use of a co-solvent did not show a statistically significant difference in the total flavonoid content of the extracts compared to the SCCO₂ method ($P < 0.05$), the use of pure CO₂ resulted in the

highest flavonoid content. Therefore, our terebinth extracts were thought to consist of flavonoids with a more complex polyphenolic structure, and due to their hydrophobic nature, higher total flavonoid content was observed in SCCO₂ and SCCO₂-EtOH extractions ($P < 0.001$).

Total Anthocyanin Contents of Extracts

The rising temperature levels create anthocyanin volatility, which results in degradation before extraction, according to previous studies about thermolabile compound extraction (De Barros et al., 2024). The highly polar nature of anthocyanins leads them to exist mainly in internal cell layers, where they reside in epidermal tissue and peripheral mesophyll cells. The polar nature of anthocyanins makes SC-O₂ extraction less effective because this method excels at extracting non-polar compounds. The limited capacity of SCCO₂ to dissolve polar compounds may result in poor extraction efficiency for polar compounds such as anthocyanins. Previous research (Silva et al., 2017) has stressed the need to use a co-solvent like ethanol for extracting polar components.

In contrast, the use of ultrasonic waves breaks down cellular structures, which enables better anthocyanin release from cells. The combination of ultrasonic waves with polar solvents such as ethanol or water enhances anthocyanin solubility, which produces better extraction yields. The US extraction method operates at low temperatures, which protects sensitive compounds from thermal degradation. The volatility of anthocyanins increases at high temperatures, thus causing their degradation before extraction, so thermal balance must be strictly maintained for sensitive compounds (Chemat et al., 2017). Many researchers studying anthocyanins have chosen to limit their extraction temperatures to 40°C because anthocyanins remain sensitive to heat even though higher temperatures would be beneficial for extracting other phenolic compounds (Paes et al., 2014; Paula et al., 2014; Silva et al., 2017). The anthocyanin contents obtained in this study, consistent with the literature, show that the US extraction method (5.01 ± 0.49 mg TAC / 100 g DM) provided a statistically significantly higher anthocyanin content compared to SCCO₂ (0.64 ± 0.06 mg TAC / 100 g DM) and SCCO₂-EtOH (0.16 ± 0.01 mg TAC / 100 g DM) methods ($P < 0.001$) (Figure 5). In particular, the low anthocyanin content obtained with the SCCO₂ method is thought to be due to the high temperature of 50°C applied in the study. The results show that anthocyanins are highly sensitive to heat and that high temperatures are detrimental to extraction efficiency. This shows that low temperature techniques are important in the extraction of thermolabile compounds and that ultrasonic assisted extraction, by controlling temperature, is beneficial in retaining the biological activity of anthocyanins.

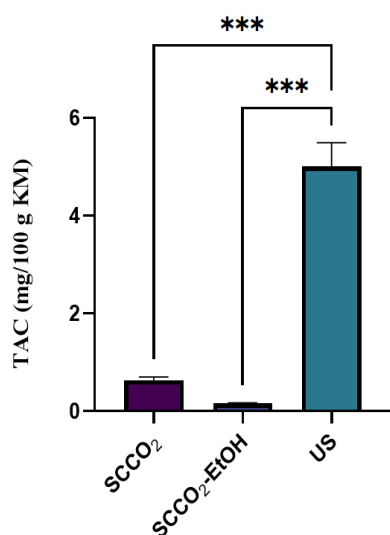


Figure 5. The total anthocyanin contents of terebinth fruit extracts obtained by different polarity extraction fluids. Data are represented as mean \pm standard deviation. *** $P < 0.001$.

The Antioxidant Activities of Extracts

In this study, the antioxidant activity of terebinth extracts obtained from different extraction methods was determined using the DPPH radical scavenging test, and the results were expressed as percentage inhibition (Figure 6). The free radical scavenging activities of the extracts obtained by SCCO₂, SCCO₂-EtOH, and US extraction methods were found to be $65.49 \pm 5.50\%$, $57.40 \pm 4.90\%$, and $60.79 \pm 5.10\%$ inhibition, respectively. The antioxidant capacities of the extracts derived from *P. terebinthus* showed no statistically significant difference ($P > 0.05$).

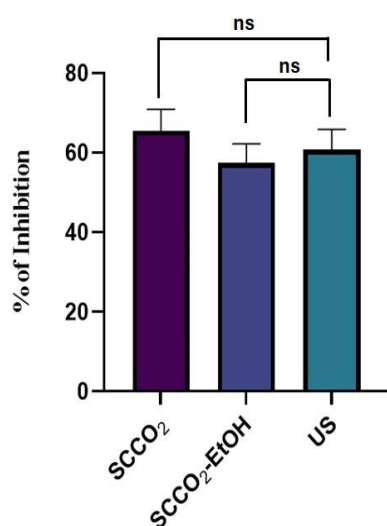


Figure 6. DPPH radical scavenging activities of terebinth fruit extracts obtained by different polarity extraction fluids. Data are represented as mean \pm standard deviation. ns: non-significant ($P > 0.05$).

In our study, although the total phenolic and flavonoid contents of *P. terebinthus* extracts obtained using different extraction methods varied significantly, no statistically significant difference was found between their antioxidant activities measured by DPPH. This could be explained by several factors: (i) since the DPPH assay measures the electron transfer mechanism via

only a single radical species, it may not equally reflect the antioxidant effects of different structural phenolic compounds; (ii) some phenolic compounds may exhibit strong antioxidant activity while others may be weak, which may cause differences in total amounts not to fully reflect functional effects; (iii) synergistic or antagonistic effects may exist between the compounds in the extracts, resulting in similar antioxidant results despite differences in total phenolic content (Amangeldinova et al., 2024); and (iv) antioxidant activities measured at levels around 60% and close to each other in all extractions suggest that the effect may have reached a certain saturation, and therefore, no statistical difference was observed. Therefore, we believe that our results are consistent with the literature (Amangeldinova et al., 2024; Farahmandfar et al., 2017; Urbonavičienė et al., 2021), which indicates that differences in extraction methods may not always directly reflect DPPH-based antioxidant activity.

Conclusion

The effect of using ethanol co-solvent as a supercritical fluid extraction parameter on the total phenolic, total flavonoid, anthocyanin contents, and antioxidant activities of the extracts of the *P. terebinthus* obtained supercritically was evaluated by comparing with the ultrasonic aqueous extract. Overall, SFE can be a viable alternative for obtaining bioactive compounds from *P. terebinthus*, apart from their culinary and fragrant use.

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Author Contributions

KO: Investigation, Methodology, Data Curation, Conceptualization, Writing -review and editing; EIA: Formal Analysis, Investigation, Methodology, Visualization and Writing -original draft; EYO: Funding Acquisition, Project Administration, Resources, Writing -review and editing

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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