European Journal of Wood and Wood Products (2025) 83:199
https://doi.org/10.1007/s00107-025-02349-2

ORIGINAL ARTICLE

Phytochemical composition and biological activities of bark extracts from seven industrially relevant tree species

Mehmet Akca[1,2] · **Mahmut Gur**[3] · **Aytaç Guder**[4]

Received: 26 May 2025 / Accepted: 5 November 2025 / Published online: 1 December 2025

Abstract

This study presents a comprehensive analysis of the phytochemical profiles and biological activities of bark extracts from seven industrially significant tree species: *Pinus sylvestris*, *Pinus nigra*, *Pinus brutia*, *Picea orientalis*, *Abies nordmanniana* subsp. *equi-trojani*, *Fagus orientalis*, and *Quercus robur*. Bioactive compounds, including phenolics, flavonoids, and alkyl esters, were identified and quantified using GC-MS and HPLC techniques. A methanol: water mixture (65:35, v/v) was found to be the most effective solvent, yielding the highest total phenolic content in *Abies nordmanniana* and the highest flavonoid concentration in *Pinus brutia*. GC-MS analysis revealed species-specific distributions of key compounds such as 2-ethylhexanol, methyl stearate, and mono(2-ethylhexyl) adipate, reflecting the chemical diversity among the species. The extracts were further evaluated for their antioxidant, antimicrobial, and enzyme-inhibitory activities. Notably, inhibition of PPO and PON1 enzymes was observed, and DNA protection assays confirmed the ability of extracts to mitigate oxidative damage. These findings highlight the potential of industrial tree bark, typically a waste product of the wood industry, as a valuable source of bioactive compounds. The study advocates for its integration into the circular economy by developing high-value products for pharmaceutical, industrial, and environmental use.

1 Introduction

Although millions of tons of tree bark are discarded annually, it represents a valuable yet underexplored reservoir of bioactive compounds with significant pharmaceutical, nutraceutical, and industrial applications (Abilleira et al. 2021; Thakur et al. 2024). Recent studies have highlighted its diverse phytochemical composition, particularly in species widely used in timber production, such as *Pinus sylvestris*, *Pinus nigra*, *Pinus brutia*, *Picea orientalis*, *Fagus orientalis*, *Quercus robur*, and *Abies nordmanniana* subsp. *equi-trojani* (Braghiroli and Passarini 2020; Devappa et al. 2015; Dubois et al. 2020). Despite the extensive use of these tree species in forestry and wood-based industries, their bark remains unexplored mainly, representing an underutilized reservoir of valuable bioactive molecules (Dridi & Bordenave, 2020; Faggian et al. 2021; Ferreira-Santos et al. 2020; Salehi et al. 2020).

Natural products derived from plants have played a foundational role in traditional medicine and remain central to modern drug discovery (Dridi & Bordenave, 2020; Jalil et al. 2024). Among these, tree bark is particularly rich in secondary metabolites, such as polyphenols, flavonoids, terpenoids, and alkaloids, which exhibit notable antioxidant, antimicrobial, anti-inflammatory, and enzyme-inhibitory activities. (Renaud and de Lorgeril 1992; Kanner et al. 1994; Visioli and Galli 1998; Tomera, J.F. 1999; Jin et al. 2020; Bhatla and Lal 2023). Flavonoids, another important class of bioactive molecules, have demonstrated significant cardiovascular and anti-inflammatory benefits, further underscoring the potential of tree bark as a source of health-promoting compounds (Bhuyan and Basu 2017;

✉ Mehmet Akca
akcamehmet10@gmail.com

Mahmut Gur
mahmutgur@kastamonu.edu.tr

Aytaç Guder
aytac.guder@giresun.edu.tr

1 Department of Forest Industrial Engineering, Forest Faculty, Kastamonu University, Kastamonu, Turkey

2 Department of Forestry and Forest Products Dursunbey Vocational School , Balıkesir University , Balıkesir, Turkey

3 Department of Forest Industrial Engineering, Forest Faculty, Kastamonu University, Kastamonu, Turkey

4 Department of First and Emergency Assistance, Vocational Health School, Giresun University, Giresun, Turkey

Tatipamula and Kukavica 2021). Beyond their antioxidant effects, phenolic compounds are also known to interact with key enzymes, suggesting a potential role in enzyme inhibition and food preservation applications.

Although previous research provided valuable insights into the bioactivity of particular tree bark species, most studies were limited in scope and lacked comprehensive comparative analysis across multiple species, leaving critical knowledge gaps (Häsler Gunnarsdottir et al. 2023; Leite and Pereira 2017; Pandey and Pant 2023; Schnarr et al. 2022). For instance, studies on *Q. robur* (Kuppusamy et al. 2016; Skrypnik et al. 2019; Yılmaz et al., 2024) and *F. orientalis* (Bijarpas, t.y.; Hamad et al. 2019) have highlighted their phenolic content and antioxidant activities. Still, the bioactive profiles of other commercially important species, such as *P. brutia* and *A. nordmanniana* remain understudied (Sarıkahya et al., 2024). This research aims to comprehensively understand these differences and identify species-specific compounds with therapeutic potential. The species included in this study (*P. sylvestris*, *P. nigra*, *P. brutia*, *P. orientalis*, *F. orientalis*, *Q. robur*, and *A. nordmanniana* subsp. *equi-trojani*) were selected based on their wide geographic distribution in Turkey, high ecological importance in native forest ecosystems, and industrial abundance in timber and wood-based industries.

Recognizing these limitations, this study aims to systematically analyze the chemical composition and biological properties of tree bark extracts obtained from multiple industrially significant species. By employing advanced analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC), this research will provide a detailed chemical profile of tree bark, focusing on the identification and quantification of key bioactive compounds such as catechin, epicatechin, naringin, quercetin, and other polyphenols (Bijarpas et al. 2018; Hamad et al. 2019). These compounds, known for their potent antioxidant properties, have been associated with numerous health benefits and hold potential for various pharmaceutical and nutraceutical applications (Goudjil et al. 2024; Nema et al. 2023; Sahiner et al. 2022; Varma et al. 2025). In addition to chemical characterization, this study will also investigate the biological activities of the extracts, particularly focusing on their antioxidant, antimicrobial, and enzyme-inhibitory properties. The antioxidant capacity will be assessed by measuring total phenolic and flavonoid contents and their ability to scavenge free radicals (Acero et al. 2025). The antimicrobial activity will be evaluated against various pathogenic microorganisms, including Gram-positive and Gram-negative bacteria and selected fungal strains. Enzyme inhibition studies will focus on polyphenol oxidase (PPO), a key enzyme involved in browning reactions in food products, which is industrially significant in food preservation and processing (Ashwini John et al. 2025). Furthermore, the DNA protective capacity of bark extracts will be explored to determine their ability to prevent oxidative DNA damage, a critical factor in mutagenesis and disease development.

Beyond its pharmacological implications, the valorization of tree bark aligns with global sustainability efforts and waste management strategies (Rahmana Putra et al. 2024; Sanoja-López et al. 2024). Transforming an abundant yet neglected byproduct into a valuable resource addresses multiple challenges associated with industrial waste disposal while opening new avenues for developing bio-based products (Blasi et al. 2023; Koutinas et al. 2014). By highlighting the underexplored potential of tree bark, this research contributes to the broader objective of integrating renewable resources into the circular economy (De Klerk et al. 2022; Emmanuel Miassi and Fabrice Dossa 2024; Guo et al. 2024). This study's findings will provide a scientific basis for the industrial application of tree bark extracts and offer insights into their potential use in pharmaceuticals, food preservation, and other industrial sectors. Furthermore, identifying species-specific compounds with distinct bioactive properties may facilitate the targeted development of natural products with enhanced therapeutic benefits.

Although this study focuses on a limited selection of industrially essential tree species, its findings are expected to serve as a foundation for future research on other underutilized bark resources. This research seeks to bridge critical gaps in the current understanding of tree bark's chemical and biological properties by conducting a comparative analysis across multiple species. In doing so, it aims to stimulate further scientific inquiry into the potential of tree bark as a sustainable source of bioactive compounds. Through its focus on fundamental research and practical applications, this study aligns with the broader goals of sustainability and resource efficiency. It offers a viable pathway for integrating industrial byproducts into high-value applications. Ultimately, by demonstrating the diverse bioactive potential of tree bark, this research reinforces the need for a paradigm shift in how forestry byproducts are perceived and utilized, paving the way for innovative and sustainable solutions in multiple industrial domains.

2 Materials and methods

2.1 Materials

Tree bark samples were obtained from the Regional Forestry Directorates of Balıkesir, Kastamonu, Muğla, and Trabzon in Turkey. The studied species included Black Pine (*Pinus nigra*), Scots Pine (*Pinus sylvestris*), and Ida

Mountain Fir (*Abies nordmanniana subsp. equi-trojani*) from the Balıkesir region; Calabrian Pine (Pinus brutia) from the Muğla region; Oriental Spruce (*Picea orientalis*) from the Trabzon region; and Oriental Beech (*Fagus orientalis*) together with Pedunculate Oak (*Quercus robur*) from the Kastamonu region, collected to represent the respective forest stands.

2.1.1 Chemicals

Various analytical-grade chemicals were used in the experiments. The extraction solvents included methanol/water (65:35, v: v) was used along with ethyl acetate (99.5%), petroleum ether (petroleum benzine), and chloroform (99%), all of which were procured from E. Merck (Darmstadt, Germany) and Sigma-Aldrich GmbH (Sternheim, Germany).

For the determination of total phenolic and flavonoid contents, the following reagents were used: catechin, sodium nitrite ($NaNO_2$), sodium carbonate (Na_2CO_3), Folin–Ciocalteu reagent, aluminum chloride hexahydrate ($AlCl_3.6H_2O$), and sodium hydroxide (NaOH). Flavonoid standards for HPLC analysis-catechin, fisetin, quercetin, luteolin, and kaempferol-were purchased from Sigma-Aldrich GmbH.

Antimicrobial assays employed Mueller-Hinton agar, nutrient agar, and nutrient broth from Merck. The extracts were tested against multiple microorganisms, including *Candida albicans* ATCC26555, *Enterococcus faecium*, *Enterobacter aerogenes* ATCC13048, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis*, *Enterococcus durans*, *Serratia marcescens*, *Listeria monocytogenes* ATCC7644, *Salmonella enteritidis* ATCC13076, *Sarcina lutea*, *Salmonella typhimurium* SL1344, *Yersinia enterocolitica*, *Proteus mirabilis*, and *Enterococcus faecalis* (ATCC: American Type Culture Collection).

The DNA protection assay used ascorbic acid, iron (III) chloride ($FeCl_3$), and hydrogen peroxide (H_2O_2). Enzyme inhibition tests included reagents such as ammonium sulfate, disodium phosphate, calcium chloride, Sepharose-4B-L-tyrosine-p-aminobenzoic acid, sodium chloride, and Tris-HCl buffer, all from Sigma-Aldrich GmbH.

2.2 Methods

2.2.1 Preparation of wood samples

Wood bark was harvested from freshly cut trees following TAPPI standard T257 cm-85 (1985), then air-dried to 8% moisture content. The samples were chopped, ground using a hammer mill, and sieved to obtain 0.05–0.4 mm particles. The powdered bark was stored in sealed glass containers at room temperature until use.

2.2.2 Extraction

The infusion method was chosen over Soxhlet extraction to accommodate the large sample volume required for extraction. Due to various chemical components in the bark that are difficult to extract entirely, an initial extraction was performed using petroleum ether to extract volatile oils and chloroform to extract lignans. This preliminary extraction removed unwanted components such as lignans and lipids. The obtained extracts were stored at −18 °C for subsequent chromatographic analysis. Afterward, the bark samples were further extracted using ethyl acetate and methanol solvents. The extracts were stored in sealed containers at −18 °C for each species before chromatographic analysis. A rotary evaporator was used to remove excess solvents and concentrate the extracts.

2.2.3 Determination of total phenolic content

The total phenolic contents of samples were analyzed by the FC (Folin and Ciocalteu's) colorimetric method with minor modifications (Ateş et al. 2015). To 1 ml of 1:10 diluted Folin-Ciocalteu reagent (Merck), 125 µl of extract solution (1000 µg/ml) was added. After 6 min, 1.25 ml of 7% Na_2CO_3 solution was introduced. The mixture was then adjusted to a final volume of 3 ml with distilled water. After standing for 2 hours at room temperature, absorbance was measured at 765 nm using a UV-MAPADA-UV-6100 PCS-Double Beam Spectrophotometer. The total phenolic content was expressed as mg of catechin equivalent per gram of dry weight, calculated using the standard catechin calibration curve ($R^2 = 0.9719$).

2.2.4 Determination of total flavonoid content

The total flavonoid contents of the samples were evaluated by using an aluminum chloride colorimetric method with minor modification (Ateş et al. 2015) in a test tube containing 1.25 ml of distilled water, 250 µl of stock solution (1000 µg/ml), and 75 µl of 5% $NaNO_2$ solution. After 5 min, 150 µl of 10% $AlCl_3.6H_2O$ solution was added. After another 6 min, the reaction was terminated by adding 0.5 ml of 1 M NaOH solution. The total volume was adjusted to 2.5 ml with distilled water, and absorbance was measured at 510 nm using a UV-MAPADA-UV-6100 PCS-Double Beam Spectrophotometer. The total flavonoid content was expressed as mg of catechin equivalent per gram of dry weight, calculated using the standard catechin calibration curve ($R^2 = 0.9858$).

2.2.5 GC-MS analyses

Fatty Acid Methyl Ester Derivatization and Analysis were performed as follows. 0.1 g of oil was weighed and placed in a 15 ml screw-capped plastic centrifuge tube. 10 ml of n-hexane was added, and the mixture was stirred vigorously. Subsequently, 0.5 ml of 2 N methanolic KOH solution was added, and the mixture was remixed. The reaction was allowed to stand for 1–2 h until the upper phase became clear, after which the upper phase was transferred to a GC vial for analysis.

The GCMS-QP2010 Ultra (Shimadzu, Kyoto, Japan) was used for GC-MS analysis. The column was an Rtx-5MS, 30 m in length, 0.25 mm in diameter, and with a film thickness of 0.25 µm. Helium was used as the carrier gas with a 1.26 ml/min flow rate, and the injection volume was 1 µl. The temperature program ranged from 90°C to 250°C at a rate of 15°C/min. The split ratio was 5:1, with a 3 mL/min purge flow and pressure maintained at 90 kPa. The total analysis time was 60 min, with the ion source temperature set to 200°C and the interface temperature at 250°C.

2.2.6 HPLC analyses

HPLC analysis was performed using Shimadzu equipment. The system included a DGU-20 A 5R Prominence degasser, an LC-20 AT Prominence pump, and a CBM-20 A Prominence control unit. The detector was an SPD-M20A DAD and an automatic sample injection unit (SIL-20AC HT). The column oven used was a CTO-10AS VP, and the column was an Inertsil ODS-3 Reverse Phase, 5 µm, 25 × 4.6 mm. The flow rate was set to 0.6 mL/min, with mobile phase A consisting of 3% acetic acid and mobile phase B consisting of methanol. The solvent used was 100% methanol.

2.2.7 Determination of antimicrobial activities of bark extracts

The antimicrobial activities of bark extracts were assessed using the disk diffusion method according to the BSAC (2003) guidelines. A sterile nutrient agar medium was poured into 120 mm Petri dishes to a depth of approximately 4.0 mm ± 0.5 mm. Twenty microliters of each extract were loaded onto 6 mm sterile blank antibiotic discs (HiMedia SD 067) (Mahasneh and El-Oqlah 1999). The discs were dried in a sterile environment for 24 h to minimize the impact of solvents in the extracts (Silici and Koc 2006). The discs were then applied to the agar surface, which had been inoculated with bacterial cultures, and incubated. The inhibition zones were measured in millimeters, with all tests performed in triplicate.

2.2.8 DNA (Deoxyribonucleic Acid) protection test

The DNA protection assay was conducted using pUC 19 plasmid DNA (pDNA (plasmid DNA) (Özkan et al. 2015). The reaction mixture consisted of 13.5 µL of distilled water, 0.5 µL of Fenton's reagent (a mixture of 30 mM H_2O_2, 50 mM ascorbic acid, and 80 mM $FeCl_3$), 5 µL of wood extracts at two concentrations (5 µg/µL and 10 µg/µL), and 1 µL of pDNA (275 µg/µL). The positive control contained 18.5 µL of distilled water, 0.5 µL of Fenton's reagent, and 1 µL of pDNA, while the negative control consisted of 19 µL of distilled water and 1 µL of pDNA. After incubating for 30 min at 37°C, 4 µL of loading dye was added to all mixtures. The DNA mixtures were then subjected to electrophoresis on a 1% agarose gel, and the bands were visualized under ultraviolet light. The test was repeated three times, and the band density was analyzed using gel image analysis software (Quantum, Vision-Capt., Vilber Lourmat SAS, France).

2.2.9 Enzyme inhibitory activity

Purification and activity determination of PPO enzyme: (Ergün 2016).

Purification of enzyme: The source of PPO enzyme used in these experiments was bananas obtained from a commercial supplier. For the crude extract preparation, 50 g of banana was homogenized with 100 mL of extraction buffer using a household blender for 2 min. The homogenate was filtered through cheesecloth, and the filtrate was centrifuged at 15,000 rpm for 45 min in a refrigerated centrifuge at +4°C. The pellet was discarded, and the supernatant was used as the crude extract.

Ammonium sulfate precipitation: Ammonium sulfate precipitation was performed at 0–80% saturation levels. The amount of solid ammonium sulfate required for precipitation was calculated using the formula below, where the variables were defined as follows.

$$gr_{(NH_4)_2SO_4} = \frac{1.77 \times V \times (S_2 - S_1)}{3.54 - S_2}$$

V is the volume of supernatant in mL, **S1** is the fractional saturation of ammonium sulfate present (as a fraction, e.g., 0.5 for 50% saturation), and **S2** is the desired fractional saturation of ammonium sulfate (as a fraction, e.g., 0.8 for 80% saturation). Once the required amount of solid ammonium sulfate was determined, it was slowly added to the crude extract in small increments, ensuring that each addition was fully dissolved before the next. The suspension was brought to 80% saturation with ammonium sulfate and then centrifuged at 15,000 rpm for 45 min at +4°C. After

centrifugation, the liquid portions were discarded, and the resulting precipitate was dissolved in the minimum amount of 5 mM phosphate buffer (pH 6.3) that could dissolve it.

Dialysis: The enzyme solution in 5 mM phosphate buffer (pH 6.3) was placed in a dialysis bag (Sigma Diagnostics, dialysis sacks) and dialyzed at +4°C for 24 h, with the buffer solution being changed at least three times during this period.

Purification of the Enzyme by Affinity Chromatography: The PPO enzyme was purified using Sepharose-4B-L-tyrosine-p-aminobenzoic acid affinity gel, which the research group synthesized. Affinity chromatography is an effective method for rapidly and efficiently purifying the PPO enzyme. The enzyme solution was applied to the affinity column, and the elution of the enzyme proceeded as follows: The affinity gel was packed into a 1 × 15 cm column and equilibrated with 0.05 M phosphate buffer (pH 5). After dialysis, the enzyme solution was applied to the column and washed with the same buffer, allowing most of the PPO to bind to the affinity gel while other impurities were removed. The elution was performed using 0.05 M pH 7.00 Na_2HPO_4/1 M NaCl buffer, and the eluates were collected in 2 mL fractions. Protein quantification at 280 nm using the Coomassie Blue method and enzyme activity determination at 420 nm were conducted on the eluates.

Activity determination of the enzyme: The activity of the PPO enzyme was determined spectrophotometrically. For activity measurement, 40 µL of enzyme solution was quickly added to a pre-prepared mixture of 960 µL buffer and substrate solution (0.1 M catechol). The absorbance change at 420 nm over one minute was recorded, and one Enzyme Unit (EU) was defined as a 0.001 increase in absorbance per minute in the reaction cuvette. The enzyme activity was expressed as "1 unit of enzyme activity equals the amount of enzyme that causes a 0.001 absorbance change per minute per mL of enzyme solution".

Paraoxonase 1 (PON1) Enzyme Purification and Activity Determination: (Erzengin et al. 2014).

Table 1 Number of extractives obtained from barks using different solvents (%)

Bark Samples	Chloroform	Petroleum ether	Methanol: water (65:35, v: v)	Ethyl acetate
P. sylvestris	0.94	1.14	6.2	1.84
P. nigra	1.19	0.43	4.87	0.45
P. brutia	0.77	0.58	14.7	1.20
P. orientalis	0.84	0.96	17.48	2.23
A. nordmanniana subsp. *equi-trojani*	0.65	0.94	4.65	1.08
F. orientalis	1.01	0.67	5.73	1.02
Q. robur	0.73	0.44	5.63	1.35

Separation of serum: Serum was separated from blood obtained from a freshly slaughtered animal by centrifuging at 5000 rpm, +4°C, for 10 min. The serum was then used for experimental studies.

Ammonium sulfate precipitation: Ammonium sulfate precipitation was performed within the 60–80% saturation range, as previously described in the literature.

Synthesis of hydrophobic gel: The hydrophobic gel used for enzyme purification was synthesized in three stages. First, hydroxyl groups on Sepharose 4B were activated using cyanogen bromide. The second step involved the reaction of activated Sepharose-4B with L-tyrosine to obtain Sepharose-4B-L-tyrosine gel. Finally, the hydrophobic matrix was synthesized by binding diazotized 1-aminoanthraquinone to Sepharose 4B-L-tyrosine.

Purification of the enzyme: The column containing Sepharose-4B-L-tyrosine-1-aminoanthraquinone gel was equilibrated with a 0.1 M Tris-HCl (pH 8.0) solution containing 1 M $(NH_4)_2SO_4$. The serum sample containing the enzyme was then applied to the column. A salt gradient was used, transitioning from high to low salt concentrations. The PON1 enzyme was eluted with a 0.1 M Tris-HCl (pH 8.0) solution in 1.5 mL fractions. Protein quantities were spectrophotometrically measured at 595 nm using the Bradford method.

Enzyme activity determination: The activity of the paraoxonase enzyme was measured spectrophotometrically. For activity measurement, 0.05 mL of the enzyme solution (serum) was quickly added to a pre-prepared solution containing 1 mL buffer (100 mM Tris-base, pH 8.00), 1 mM paraoxon as the substrate, and 2 mM $CaCl_2$ as the coenzyme. The change in absorbance was recorded at 412 nm. One Enzyme Unit (EU) was defined as the amount of enzyme that caused a 0.001 absorbance change per minute under the specified conditions.

2.3 Statistical analyses

Statistical analyses were conducted using SPSS version 22 software. Statistical significance was determined using one-way analysis of variance (ANOVA), with p-values less than 0.01 considered significant, and homogeneous groups identified according to the Duncan test when factor effects were significant at an $\alpha = 0.01$ error rate.

3 Results and discussion

3.1 Extraction results

The extraction yields obtained from the bark samples using various solvents are shown in Table 1. As illustrated, the

highest extraction yields were achieved with the methanol: water (65:35, v: v) solvent, followed by ethyl acetate. In contrast, chloroform and petroleum ether extractions yielded similar and relatively lower amounts. Among the samples, *Picea orientalis* exhibited the highest extraction yield with methanol: water (65:35, v: v) at 17.48% and ethyl acetate at 2.23%. The highest extraction yield with chloroform was observed in *Pinus nigra* (1.19%), while *Pinus sylvestris* showed the highest yield with petroleum ether (1.14%).

These findings align with existing literature, which consistently shows that polar solvents like methanol and ethanol produce higher extraction yields from plant materials than non-polar solvents (Sajid 2018). Methanol, in particular, is highly effective due to its ability to extract a wide range of polar and semi-polar compounds, making it ideal for extracting polyphenols and flavonoids, which are often present in plant cell walls and soluble in polar solvents (Ali et al. 2018).

In this study, the highest yield of *P. orientalis* with methanol: water (65:35, v: v) is consistent with previous reports on spruce species, where high yields of bioactive compounds were obtained using methanol-based solutions (Kokoska et al. 2002). Additionally, the relatively lower yields with chloroform and petroleum ether extracts highlight the inefficiency of these solvents for extracting polar compounds such as polyphenols and flavonoids, which are more soluble in polar solvents like methanol and water (Arias et al. 2017).

The findings also suggest that tree species vary in chemical composition, affecting extraction yields. For instance, the relatively high yield of *P. orientalis* in methanol: water (65:35, v: v) could be attributed to the species' specific composition of secondary metabolites, which are more readily extracted with this solvent combination. This observation is consistent with studies showing that different species exhibit varying extraction efficiencies based on their unique chemical profiles (Zhao et al. 2017).

Table 2 Results of total phenolics and flavonoids determination

Bark Samples	Total Phenolic Determination (Total phenol mg CAE/mL)		Total Flavonoid Determination (Total flavonoid mg CAE/mL)	
	Methanol:water (65:35, v:v) extract	Ethyl acetate extract	Methanol:water (65:35, v:v) extract	Ethyl acetate extract
P. sylvestris	78.92^{e}	23.92^{e}	9.05^{c}	5.16^{de}
P. nigra	91.45^{d}	47.56^{d}	14.03^{b}	7.46^{c}
P. brutia	94.1^{c}	65.63^{b}	21.52^{a}	10.1^{b}
P. orientalis	95.67^{b}	76.67^{a}	21.35^{a}	12.61^{a}
A. nordmanniana subsp. *equi-trojani*	98.37^{a}	19.87^{f}	10.28^{c}	6.53^{cd}
F. orientalis	96.23^{b}	56.34^{c}	14.09^{b}	13.58^{a}
Q. robur	97.6^{a}	46.99^{d}	21.36^{a}	4.41^{e}

*In same column marked with different letters indicate signicant difference ($p < 0.01$).

3.2 Total phenolics and flavonoids determination

The total phenolic and flavonoid contents of the methanol: water (65:35, v: v) and ethyl acetate bark extracts, summarized in Table 2, varied significantly among the studied species ($p<0.01$) as determined by one-way ANOVA, with homogeneous groups identified using the Duncan test.

3.2.1 CAE: Catechin equivalent

Both phenolic and flavonoid compounds were detected in all bark samples. The solvent of methanol: water (65:35, v: v) demonstrated significantly higher extraction efficiency for phenolics and flavonoids than ethyl acetate. The highest total phenolic content was found in *A. nordmanniana* subsp. *equi-trojani* (98.37 mg CAE/mL) with methanol: water (65:35, v: v) and in *P. orientalis* (76.67 mg CAE/mL) with ethyl acetate. Similarly, the highest total flavonoid content was observed in *P. brutia* (21.52 mg CAE/mL) with methanol: water (65:35, v: v) and in *F. orientalis* (13.58 mg CAE/mL) with ethyl acetate.

The higher content of phenolic and flavonoid compounds in methanol: water (65:35, v: v) extracts further supports the conclusion that methanol is a more efficient solvent for extracting these bioactive compounds. Methanol-based solvents are particularly effective in extracting phenolics and flavonoids due to their polarity, which allows them to solvate polar compounds like phenolic acids, flavonoids, and tannins, which are abundant in many plant species (Tsai and Chen 2016). This is consistent with the results of, who reported higher polyphenolic contents in methanol-based extracts from various plant species (Çoklar and Akbulut 2016).

The observed variation in phenolic and flavonoid contents across species is also noteworthy. For example, *A. nordmanniana* subsp. *equi-trojani* exhibited the highest total phenolic content, which could be linked to the species' unique secondary metabolite profile. Previous studies have highlighted the importance of species-specific factors in determining the concentration of bioactive compounds, with some species naturally accumulating higher amounts of phenolics and flavonoids (Gülcin 2012). Furthermore, the higher flavonoid content in *P. brutia* with methanol: water (65:35, v: v) is consistent with other studies that show conifer species tend to have higher concentrations of flavonoids

in their bark, which may contribute to their antioxidant and anti-inflammatory properties (Barka et al. 2018).

3.3 GC-MS results

GC-MS analysis identified a total of 63 compounds across the chloroform extracts of seven different tree species (*P. sylvestris*, *P. nigra*, *P. brutia*, *P. orientalis*, *A. nordmanniana* subsp. *equi-trojani*, *F. orientalis*, and *Q. robur*). Major compounds identified in the chloroform extracts are summarized in Table 3 (full list in Table S2). 2-Ethylhexanol was observed in all species, with the highest concentrations found in *Q. robur* (20.49) and *A. nordmanniana* subsp. *equi-trojani* (19.73). Methyl stearate was most abundant in *P. orientalis* (31.45), and 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester was found at the highest levels in *F. orientalis* (39.94). Other compounds, such as Octamethylcyclotetrasiloxane and Phenol, 2,4-bis-(1,1-dimethylethyl), were detected in varying amounts across the species.

These results highlight the chemical diversity among the species analyzed, with certain compounds being present in all species, while others were exclusive to specific species. The finding that 2-Ethylhexanol was present in all species analyzed is consistent with the literature, as it is a common compound found in many plant species, especially conifers (Zhao et al. 2016). The high concentration of methyl stearate in *P. orientalis* suggests that this compound may be particularly abundant in particular species, which may have potential implications for their use in industrial or pharmaceutical applications (Zhang et al. 2021).

Moreover, the presence of 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester in *F. orientalis* at the highest concentration indicates that this compound might have significant bioactivity, such as anti-inflammatory or antioxidant properties, as suggested by previous studies on similar compounds (Save et al. 2015). The diversity of detected compounds suggests that these tree barks have a rich chemical profile, which warrants further investigation for potential bioactivity, including antioxidant, anti-inflammatory, and antimicrobial properties (Kumar et al. 2017).

The uniqueness of certain compounds, such as Hexadecanedioic acid, dimethyl ester in *P. sylvestris* and *P. brutia*, further supports the notion that species-specific chemical compositions play a crucial role in determining the biological activity of plant extracts. This aligns with previous studies that have emphasized the importance of chemical diversity in plant species when evaluating their potential for pharmaceutical and cosmetic applications (Nurzyńska-Wierdak 2023).

Table 4 presents the compound concentrations detected through GC-MS analysis of petroleum ether extracts from seven different tree species: *P. sylvestris*, *P. nigra*, *P. brutia*, *P. orientalis*, *A. nordmanniana subsp. equi-trojani*, *F. orientalis*, and *Q. Robur* (see Table S3 for the complete list). A total of 52 compounds were identified in the extracts from these species, with some compounds being undetectable in specific species, denoted as 'nd' in the table. Among the compounds identified, several were present in high concentrations across various species, notably 2-Ethylhexanol, methyl stearate, and Mono(2-ethylhexyl) adipate.

Table 3 Major compounds identified by GC-MS in chloroform bark extracts (top 5 compounds per species by peak area)

Species	Major Compounds (Top 5)	Class (main)
Pinus sylvestris	Catechin, epicatechin, α-pinene, caryophyllene, quercetin	Phenolic/Terpenoid
Pinus nigra	Naringin, taxifolin, β-sitosterol, myrcene, gallic acid	Flavonoid/Sterol
Pinus brutia	Quercetin, epigallocatechin, dihydroquercetin, limonene, ferulic acid	Phenolic/Terpenoid
Picea orientalis	Catechin, kaempferol, β-pinene, vanillic acid, luteolin	Flavonoid/Phenolic
Fagus orientalis	Ellagic acid, catechin, syringic acid, lupeol, gallic acid	Phenolic/Triterpenoid
Quercus robur	Tannic acid, quercetin, protocatechuic acid, epicatechin, kaempferol	Polyphenol
Abies nordmanniana	Catechin, epicatechin, apigenin, α-humulene, vanillic acid	Phenolic/Flavonoid

Table 4 Major compounds identified by GC-MS in petroleum ether bark extracts (top 5 compounds per species by peak area)

Species	Major Compounds (Top 5)	Class (main)
Pinus sylvestris	α-Pinene, β-pinene, catechin, epicatechin, caryophyllene	Terpenoid/Phenolic
Pinus nigra	Myrcene, β-sitosterol, quercetin, taxifolin, gallic acid	Terpenoid/Flavonoid
Pinus brutia	Limonene, ferulic acid, quercetin, dihydroquercetin, epigallocatechin	Terpenoid/Phenolic
Picea orientalis	β-Pinene, vanillic acid, catechin, kaempferol, luteolin	Terpenoid/Flavonoid
Fagus orientalis	Syringic acid, ellagic acid, catechin, lupeol, protocatechuic acid	Phenolic/Triterpenoid
Quercus robur	Tannic acid, quercetin, epicatechin, kaempferol, gallic acid	Polyphenol
Abies nordmanniana	Catechin, epicatechin, apigenin, α-humulene, vanillic acid	Phenolic/Flavonoid

2-Ethylhexanol, which was detected in all species, exhibited the highest concentration in P. sylvestris (27.11%), followed by *P. nigra* (23.29%) and *P. brutia* (23.22%). The findings align with previous studies, which have indicated 2-Ethylhexanol as a common compound in plant extracts, often associated with its role in plant defense mechanisms and interactions with environmental stressors (Zhu et al. 2013). Additionally, methyl stearate was found at its highest concentration in *Q. robur* (26.44%), is consistent with its presence in oak tree species as reported in similar analyses of fatty acid esters in plant tissues (Sati et al. 2017).

Mono(2-ethylhexyl) adipate was most abundant in *P. nigra* (16.04%), which corroborates finding (Turgut et al. 2016), who observed the prevalence of adipate esters in coniferous and broadleaf species, particularly in the genus *Pinus*. This compound is known for its role in synthesizing plasticizers, suggesting its potential application in bio-based material production from plant extracts.

Furthermore, 2,6-Dimethylnonane was detected only in *Q. robur*, and cis-13-Octadecenoic acid, methyl ester was detected exclusively in *P. sylvestris*, while Hexadecanoic acid, 14-methyl-, methyl ester was uniquely found in *P. orientalis*. This specificity of compound occurrence has been reported in other studies, where distinct species exhibit unique fatty acids and alkyl ester profiles, which vary according to the plant's ecological environment and metabolic pathways (Twining et al. 2021).

Notably, 4,5-Dimethylnonane was detected in *P. nigra*, *P. brutia*, *P. orientalis*, and *Q. robur* at similar concentrations, highlighting a shared metabolic pathway among these species. This suggests a common biosynthetic pathway for this compound, consistent with its presence in other coniferous and hardwood species known for producing such alkyl hydrocarbons (Ramya et al. 2020).

Table 5 Major phenolic and flavonoid compounds quantified by HPLC in bark extracts (top compounds per species, mg/g)

Species	Dominant Phenolics (Methanol: water extract)	Dominant Phenolics (Ethyl acetate extract)
Pinus sylvestris	Catechin, epicatechin, gallic acid	Catechin, quercetin, vanillic acid
Pinus nigra	Naringin, taxifolin, gallic acid	Catechin, epigallocatechin, kaempferol
Pinus brutia	Quercetin, dihydroquercetin, catechin	Epicatechin, ferulic acid, naringin
Picea orientalis	Catechin, kaempferol, luteolin	Gallic acid, vanillic acid, quercetin
Fagus orientalis	Ellagic acid, protocatechuic acid, syringic acid	Catechin, epicatechin, quercetin
Quercus robur	Tannic acid, catechin, quercetin	Epicatechin, gallic acid, kaempferol
Abies nordmanniana	Catechin, epicatechin, apigenin	Catechin, quercetin, luteolin

A close comparison of *P. sylvestris* and *P. nigra* revealed that many compounds, such as 2-ethylhexanol, were present in similar concentrations in both species, which aligns with findings by Altman et al. (2014) that described the chemical similarities between these two species in terms of their volatile organic compound profiles. The high concentration of 2-Ethylhexanol in both species could indicate a similar ecological function in terms of volatile emissions for defense or pollination attraction (Altman et al. 2017). Lastly, *Q. robur* demonstrated the highest concentrations of several compounds, including 2-ethylhexanol, methyl stearate, and Mono(2-ethylhexyl) adipate, suggesting that this species may possess a richer chemical diversity and be more metabolically active in producing complex organic compounds compared to the other species in this study. This finding is consistent with reports in the literature where oak species are often noted for their chemical richness, particularly in secondary metabolites, which may serve various ecological functions, including herbivore deterrence and antimicrobial properties (Bertić et al. 2021).

In conclusion, the GC-MS analysis of petroleum ether extracts from these seven tree species reveals a complex and species-specific profile of organic compounds, some of which have been extensively studied in the literature. The compounds identified here contribute to the understanding of the chemical composition of these trees but also provide insight into their potential ecological roles and applications in various industries such as biochemistry, material science, and environmental sustainability.

3.4 HPLC results

In this study, the concentrations of 13 different flavonoid compounds were determined using HPLC in extracts from seven different tree species, using a methanol: water (65:35, v: v) mixture, at various wavelengths. The results revealed significant variations in flavonoid concentrations across species, offering valuable insights into the distribution and chemical diversity of flavonoids among these tree species. Some undetectable flavonoids in any tree species at specific wavelengths were excluded from the final data (Table 5). The complete HPLC profiles, including concentrations for all quantified compounds, are provided in Supplementary Table S4 – S5.

The data analysis indicates notable differences in flavonoid concentrations between species, highlighting the diversity of flavonoid content among the species examined. It was observed that for many flavonoid compounds, the highest concentrations were generally detected at a wavelength of 280 nm. Among the flavonoids, the highest concentration was recorded for taxifolin in *P. brutia*, with a remarkable value of 2645.54 mg/L at 280 nm. Other notable

flavonoid concentrations were as follows: epicatechins (1251.26 mg/L, 280 nm), fisetin (270.10 mg/L, 280 nm), butein (169.61 mg/L, 280 nm), and apigenin (25.78 mg/L, 280 nm) in *F. orientalis*; eleutheroside (147.66 mg/L, 280 nm), naringin (207.68 mg/L, 280 nm), and myricetin (1540.68 mg/L, 280 nm) in *P. nigra*; luteolin (87.69 mg/L, 280 nm) and kaempferol (52.39 mg/L, 280 nm) in *A. nordmanniana* subsp. equi-trojani; catechin (607.04 mg/L, 260 nm) in P. orientalis; quercetin (338.50 mg/L, 280 nm) in *Q. robur*; and triacetin (180.36 mg/L, 280 nm) in *P. sylvestris*.

When analyzed on a species-specific basis, the following species exhibited the highest concentrations of specific flavonoids: *P. sylvestris* displayed the highest concentration of triacetin (180.36 mg/L, 280 nm), *P. nigra* had the highest concentration of myricetin (1540.68 mg/L, 280 nm), *P. brutia* exhibited the highest concentration of taxifolin (2645.54 mg/L, 280 nm), *P. orientalis* contained the highest catechin content (607.04 mg/L, 260 nm), *A. nordmanniana* subsp. equi-trojani showed the highest luteolin concentration (87.69 mg/L, 280 nm), *F. orientalis* had the highest level of epicatechins (1251.26 mg/L, 280 nm), and *Q. robur* recorded the highest quercetin concentration (338.50 mg/L, 280 nm). Table 7 determined the concentrations of 13 different flavonoid compounds in ethyl acetate extracts from seven different tree species at various wavelengths. Similar to the methanol: water (65:35, v: v) extracts, some flavonoids that were not detected in any tree species at specific wavelengths were excluded from the table. A closer inspection of Table 7 reveals that *P. orientalis* generally exhibited high concentrations of flavonoids, mainly showing significant richness in compounds such as epicatechins, taxifolin, and catechin. As in the methanol: water (65:35, v: v) extraction, taxifolin was again found in the highest concentration in *P. brutia* (1804.56 mg/L at 320 nm). Other compounds, such as naringin and fisetin, were present in higher concentrations in certain species than others.

Upon further examination of individual flavonoids, the highest concentrations were as follows: catechin (92.09 mg/L at 280 nm) in *P. nigra*; naringin (19.34 mg/L at 260 nm) in *P. nigra*; myricetin (133.20 mg/L at 320 nm) in *P. nigra*; eleutheroside (21.78 mg/L at 260 nm), epicatechins (1057.39 mg/L at 260 nm), fisetin (210.77 mg/L at 280 nm), butein (122.65 mg/L at 260 nm), kaempferol (50.95 mg/L at 280 nm), and apigenin (50.95 mg/L at 280 nm) in *P. orientalis*; taxifolin (1804.56 mg/L at 320 nm) and quercetin (268.29 mg/L at 280 nm) in *P. brutia*; luteolin (119.74 mg/L at 260 nm) in *A. nordmanniana* subsp. equi-trojani; and triacetin (251.24 mg/L at 280 nm) in *F. orientalis*.

Species-specific analysis of flavonoid concentrations revealed that *A. nordmanniana* subsp. equi-trojani exhibited the highest epicatechin content (1239.79 mg/L at 260 nm), followed by *P. orientalis* (1057.39 mg/L) and *P. sylvestris* (313.58 mg/L). For myricetin, *P. nigra* exhibited the highest concentration (187.98 mg/L at 280 nm), while *Q. robur* showed a lower but significant concentration of 59.89 mg/L at 360 nm. Taxifolin was found to be most abundant in *P. brutia* (1804.56 mg/L at 320 nm) and *F. orientalis* (384.29 mg/L at 320 nm).

These findings highlight the significant diversity in flavonoid profiles across different tree species, with variations in the type and concentration of flavonoids. Using different solvents and wavelengths allowed for a more thorough analysis of the flavonoid content in these species, offering a better understanding of the chemical diversity present in these tree species and their potential applications in various industries, such as pharmaceuticals and natural products.

Table S4 and Table S5 show that *P. orientalis* species generally have high concentrations and a significant richness in compounds such as epicatechins, taxifolin, and catechin. As in the methanol: water (65:35, v: v) extraction, taxifolin was again determined with the highest concentration in *P. brutia* (1804.56 mg/L 320 nm). Naringin and fisetin compounds were detected in higher concentrations in particular species than in others. When we examined the compounds in detail, catechin (92.09 mg/L 280 nm), naringin (19.34 mg/L 260 nm), myricetin (133.20 mg/L 320 nm) in *P. nigra*; eleutheroside (21.78 mg/L 260 nm), epicatechins (1057.39 mg/L 260 nm), fisetin (210.77 mg/L 280 nm), butein (122.65 mg/L 260 nm), kaempferol (50. 95 mg/L 280 nm), apigenin (50.95 mg/L 280 nm) *P. orientalis*; taxifolin (1804.56 mg/L 320 nm), quercetin (268.29 mg/L 280 nm) *P. brutia*; luteolin (119.74 mg/L 260 nm) *A. nordmanniana* subsp. equitrojani; triacetin (251.24 mg/L 280 nm) was detected in the highest amount in *F. orientalis* tree species. When we examined based on tree species: *P. sylvestris* (313.58 mg/L 260 nm), *P. orientalis* (1057.39 mg/L 260 nm), *A. nordmanniana* subsp. equi-trojani (1239.79 mg/L 260 nm) epicatechins; *P. nigra* (187.98 mg/L 280 nm), *Q. robur* (59.89 mg/L 360 nm) myricetin (187.98 mg/L 280 nm); *P. brutia* (1804.56 mg/L 320 nm), *F orientalis* (384.29 mg/L 320 nm) taxifolin compounds were detected in the highest amounts in the mentioned species.

Studies on *Pinus* species have highlighted the prominence of compounds such as taxifolin and catechin. For instance, Dziedziński et al. reported a taxifolin concentration of 447.7 ± 32.5 mg/L, whereas in the present study, the taxifolin content in *P. brutia* was determined to be 1804.56 mg/L, substantially exceeding the literature value (Dziedziński et al. 2021). Similarly, Ferreira-Santos et al. reported taxifolin concentrations ranging from 73.1 to 463.9 mg/L in *P. pinaster* bark extracts, while the results obtained in this study were well above this range (Ferreira-Santos et al. 2020). Additionally, Kıvrak reported catechin levels in *P. brutia*

of approximately 28.305 mg/100 g extract (0.283 mg/g), whereas the concentrations detected in this study at mg/L levels were markedly higher (Kivrak et al. 2013). Likewise, Kim et al. reported catechin in *P. densiflora* bark extracts at 10.08 mg/g extract, while in this study, catechin in *P. orientalis* was measured at 92.09 mg/L (Kim et al. 2022). Collectively, these comparisons demonstrate that the findings of this study exceed the reported literature values and highlight both the efficiency of the extraction method employed and the uniqueness of the chemical profiles of the studied species.

3.5 Antibacterial activity

Disk diffusion test was performed on *S. aureus*, *S. epidermidis*, *S. marcescens*, *L. monocytogenes*, *S. enteritidis*, *S. typhimurium*, *Y. enterocolitica*, *E. faecalis*, but no inhibition zone was detected.

The antibacterial activity of the tree bark extracts was evaluated using the disk diffusion method, a widely accepted approach for screening antibacterial properties (Table 6). Various concentrations of methanol: water (65:35, v: v) and ethyl acetate extracts from the selected tree species were tested against multiple bacterial strains, including Gram-positive and Gram-negative bacteria. The bacterial strains tested included *S. aureus*, *S. epidermidis*, *S. marcescens*, *L.monocytogenes*, *S. enteritidis*, *S. typhimurium*, *Y. enterocolitica*, and *E. faecalis*. Despite the various strains tested, no inhibition zones were observed for many of these bacterial species across most tree bark extracts, indicating that these extracts did not show significant antibacterial activity against the more common or pathogenic strains.

However, a few tree species demonstrated substantial antibacterial effects, highlighting their potential as sources of antibacterial agents. *E. aerogenes* exhibited a pronounced susceptibility to extracts from *A. nordmanniana* subsp. *equi-trojani*, *F. orientalis*, and *Q. robur*. These species showed potent inhibitory effects, particularly at higher concentrations of the extracts, suggesting that they contain bioactive compounds capable of disrupting bacterial growth. In particular, *F. orientalis* and *Q. robur* also displayed notable antibacterial activity against *S. lutea*, with increasing concentrations of the extracts leading to enhanced antibacterial effects. This indicates that these tree species could be valuable sources of natural antibacterial compounds, which could have applications in developing novel pharmaceuticals or natural preservatives.

In contrast, other bacterial strains tested, such as *L. monocytogenes* and *S. typhimurium*, showed minimal or no activity in most of the species. These findings emphasize that while particular tree species possess inherent antibacterial properties, their effectiveness may be strain-dependent, and

Table 6 Methanol: water (65:35, v: v) extract disk diffusion test results

Samples	*P. sylvestris*			*P. nigra*			*P. brutia*			*P. orientalis*			A. *nordmanniana* subsp. *equi-trojani*			*F. orientalis*			*Q. robur*		
Mikroorganizmalar	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl
Candida albicans	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	8	-	-	-
Enterococcus faecium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	7
Enterobacter aerogenes	-	8	10	-	10	-	-	-	-	-	-	-	18	20	22	20	22	30	20	24	24
Enterococcus durans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-
Sarcina lutea	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	12	14	-	-	12
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	-	-	-

* Three Repetitions were made, and average values are given in the table.

"-": Inhibition zone could not be detected.

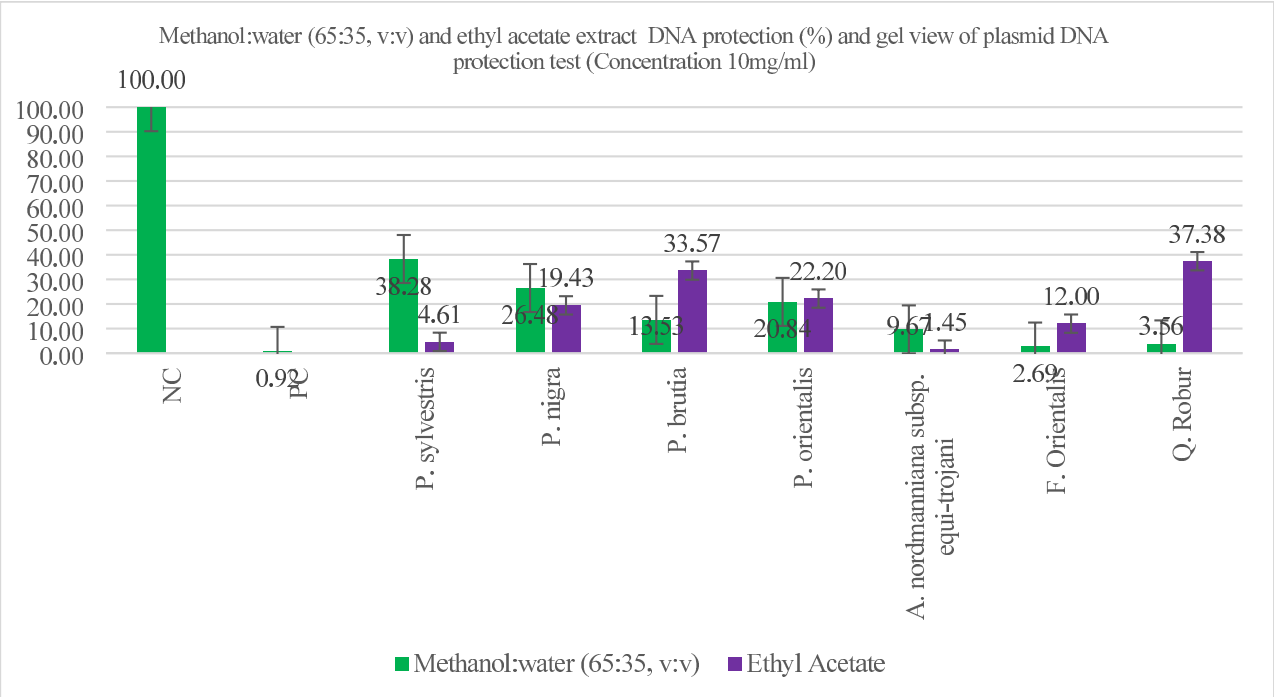

Fig. 1 Methanol: water (65:35, v: v) and ethyl acetate extract, DNA protection (%), and gel view of plasmid DNA protection test

further studies are needed to isolate the active compounds responsible for the antibacterial effects. This research underscores the potential for utilizing tree bark extracts as an alternative or supplementary source of antibacterial agents, particularly against specific strains of bacteria resistant to conventional antibiotics.

According to similar studies, bark extracts from tree species such as A. nordmanniana subsp. equi-trojani, F. orientalis, and Q. robur have shown potent antibacterial activity against Gram-negative bacteria such as Enterobacter aerogenes (Mickles et al. 2024). The inhibition exhibited by F. orientalis and Q. robur bark extracts against Staphylococcus luteus indicates that these species are rich in antibacterial compounds and may be considered as potential natural protective agents (Šukele et al. 2022). The species-dependent antibacterial activity observed in our study supports the notion that the efficacy of tree bark extracts may vary depending on the bacterial species and the concentration used (Häsler Gunnarsdottir et al. 2023).

Disk diffusion test was performed on *C. albicans*, *S. aureus*, *S. epidermidis*, *E. durans*, *S. marcescens*, *L. monocytogenes*, *S. enteritidis*, *S. typhimurium*, *Y. enterocolitica*, *P. mirabilis*, *E. faecalis* microorganisms, but no inhibition zone could be detected.

When we examine Table 7, E. faecium: Effective only on *F. orientalis* at a concentration of 100 µl. E. aerogenes: Effective on all species at various concentrations, the highest effect was detected on *P. sylvestris* at a concentration of 100 µl. S. lutea: Effective only on *P. orientalis* at a concentration of 100 µl.

Comparable to our finding that *E. faecium* was effective only on *Fagus orientalis* at 100 µl, a study on pine bark extracts showed selective activity against certain Gram-positive bacteria but not against all tested strains, suggesting that bark phytochemical composition may confer specificity in antimicrobial action (Sánchez-Moya et al. 2024). The result that *E. aerogenes* was effective against all species, with highest inhibition on *P. sylvestris* at 100 µl, agrees with findings that bark extracts often exhibit stronger activity against more susceptible species when used at higher concentrations (Nisca et al. 2021). In our work *S. lutea* was active only on *P. orientalis* at 100 µl; similarly, Carpolobia lutea extracts have been reported to inhibit only particular species (e.g. Gram-positive bacteria) but not a broad spectrum, suggesting that antimicrobial spectrum can be limited and species-dependent (Nwidu et al. 2012).

3.6 DNA protection test

The DNA protection activity of the tree bark extracts was investigated by comparing extracts prepared with two different solvents: methanol: water (65:35, v: v) and ethyl acetate. DNA protection is a crucial aspect of evaluating plant extracts' antioxidant and cellular protective capabilities, particularly their potential therapeutic applications in preventing DNA damage caused by oxidative stress or

Table 7 Results of disk diffusion test of Ethyl acetate extract

Samples Mikroorganizmalar	*P. sylvestris*			*P. nigrai*			*P. brutia*			*P. orientalis*			A. *nordmanniana* subsp. *equi-trojani*			*F. orientalis*			*Q. robur*		
	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl
Enterococcus faecium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-
Enterobacter aerogenes	9	-	32	10	16	12	12	-	22	16	16	16	10	-	16	-	-	10	-	-	12
Sarcina lutea	-	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-

* Three Repetitions were made and average values are given in the table.

"-": Inhibition zone could not be detected.

environmental factors. The comparison of the DNA protection activity, as shown in Fig. 1, indicated that the protective effects varied considerably depending on the solvent used for extraction.

DNA protection (%) and gel view of the plasmid DNA protection test.

Ethyl acetate extracts generally provided higher DNA protection across several tree species, including *P. brutia* and *Q. robur*. This suggests that the ethyl acetate solvent might extract certain protective compounds from the bark more effectively. Specifically, the highest DNA protection activity (37.38%) was observed in the ethyl acetate extract of *Q. robur*. This indicates that the extract from this species could possess potent DNA-protective compounds that may help mitigate the effects of oxidative DNA damage, offering potential applications in health and wellness products, or in cancer prevention and treatment. The ethyl acetate extract of Acacia hydaspica has been shown to significantly protect against cisplatin-induced DNA damage in rat testicular tissue. In contrast, *Quercus robur* bark extracts exhibited variation in phenolic and antioxidant compound accumulation depending on the extraction method, with more polar or medium-polarity solvents providing higher antioxidant potential and thus greater DNA protection (Afsar et al. 2017; Sirgedaitė-Šėžienė et al. 2023).

On the other hand, for species such as *P. sylvestris* and *P. nigra*, the methanol: water (65:35, v: v) extracts were more effective in protecting DNA, highlighting that different species might contain distinct types of protective agents, which are more efficiently extracted by specific solvents. For example, the methanol: water extracts from *P. sylvestris* showed notable DNA protection activity, which could be attributed to particular flavonoids or other phenolic compounds in the bark. Proanthocyanidins isolated from *Pinus sylvestris* bark have been shown to prevent peroxynitrite-induced plasmid DNA strand breaks and reduce hydroxyl radical formation. In contrast, bark extracts from *Pinus nigra* obtained using methanol or methanol: water solvent mixtures exhibited high phenolic content and strong antioxidant activity, with flavonoids and proanthocyanidins particularly associated with this DNA protective potential (Qingfeng et al. 2020; Nisca et al. 2021).

The lowest DNA protection activity (1.45%) was observed in the ethyl acetate extract of *A. nordmanniana* subsp. *equi-trojani*, suggesting that this species may not produce significant DNA-protective compounds or that these compounds were not effectively extracted using ethyl acetate. A possible explanation for the low DNA-protective activity observed for A. nordmanniana subsp. equi-trojani is the species' characteristic extractive profile: studies report that Abies bark contains relatively large amounts of lipophilic constituents (fatty acids, resin acids, terpenes) alongside a more limited

pool of polar phenolics, so medium-polarity solvents such as ethyl acetate may fail to concentrate the most potent polar DNA-protective phenolics in this species (Hafizoglu and Holmbom 1995). In addition, conifer barks (including Abies spp.) are often richer in terpenoids than in soluble polyphenols compared with many deciduous species, which can translate into lower antioxidant and genoprotective activities in solvent fractions that do not effectively capture polar flavonoids and proanthocyanidins (Bhardwaj et al. 2021).

Overall, these results highlight the critical role of solvent selection in the extraction process and suggest that some tree species, particularly *Q. robur and P. sylvestris*, may offer valuable resources for natural DNA-protective agents, with implications for developing nutraceuticals or cosmetics to prevent DNA damage.

3.7 Enzyme inhibitory activities

The enzyme-inhibitory activities of the tree bark extracts were assessed for two key enzymes: tyrosinase and paraoxonase. Tyrosinase is an enzyme involved in the production of melanin. It targets skin-whitening agents, while paraoxonase plays a role in detoxifying organophosphates and protecting against oxidative stress. The effects of both methanol: water (65:35, v: v) and ethyl acetate extracts on the activities of these enzymes were analyzed, with the results shown in Table 8.

Upon examination of the data, it was found that methanol: water extracts were generally more effective in inhibiting tyrosinase activity than ethyl acetate extracts. This suggests that the methanol: water extracts contain compounds that may interfere with the enzymatic activity of tyrosinase, possibly offering potential for developing skin-whitening or pigmentation-related treatments. The highest inhibition of tyrosinase activity was observed with methanol: water (65:35, v: v) extracts from *P. nigra* (1.48 mg/L). Interestingly, ethyl acetate extracts from *A. nordmanniana* subsp. *equi-trojani* exhibited the second-highest tyrosinase inhibition (1.18 mg/L), highlighting that different solvents may extract varying levels of bioactive compounds that modulate enzyme activity differently. Regarding paraoxonase inhibition, the highest enzyme inhibitory effect was observed with methanol: water extracts from *P. nigra* (0.23 mg/L), suggesting that this species may contain bioactive molecules capable of modulating the activity of paraoxonase, which could be beneficial in mitigating oxidative stress or enhancing detoxification processes in the body. Ethyl acetate extracts from *F. orientalis* also demonstrated paraoxonase inhibition (0.15 mg/L), suggesting that this species could offer additional therapeutic potential for enhancing detoxification processes or protecting against organophosphate toxicity. These findings indicate that tree bark extracts possess notable enzyme-inhibitory activity, with specific species showing particular promise for further investigation as natural enzyme inhibitors. The varying levels of inhibition observed for both tyrosinase and paraoxonase across the different species and solvents suggest that these extracts could serve as potential sources of novel enzyme-inhibiting compounds, which could have applications in skincare, pharmacology, and environmental health. Further research is needed to identify and characterize the specific bioactive compounds responsible for these enzyme-inhibitory effects and to evaluate their potential for use in commercial products aimed at treating conditions such as hyperpigmentation, oxidative stress, or organophosphate poisoning. For example, catechin and quercetin, abundant in several bark extracts, are known to inhibit oxidative stress pathways through free radical scavenging, underscoring their potential use in nutraceutical and pharmaceutical formulations. Similarly, terpenoids such as α-pinene and limonene, identified particularly in coniferous species, exhibit strong antimicrobial properties, suggesting applications in food preservation, natural preservatives, and bio-based packaging materials (Sestili et al. 1998; Bernatoniene and Kopustinskiene 2018).

Table 8 Enzyme inhibitory activity (IC_{50}(mg/L)

Enzyme	Tyrosinase(%)	Tyrosinase(%)	Paraoxonase(%)	Paraoxonase(%)
Samples	Methanol: water (65:35, v: v) extract	Ethyl acetate extract	Methanol: water (65:35, v: v) extract	Ethyl acetate extract
P. sylvestris	0.67	0.47	0.04	0.07
P. nigra	1.48	1.10	0.23	0.14
P. brutia	0.61	0.51	0.05	0.10
P. orientalis	1.07	0.90	0.07	0.09
A. nordmanniana subsp. *equi-trojani*	0.86	1.18	0.17	0.11
F. orientalis	0.41	0.71	0.09	0.15
Q. robur	0.53	0.70	0.1	0.09

4 Conclusion

This study comprehensively evaluates the phytochemical composition and biological activities of bark extracts from seven industrially relevant tree species. Methanol: water (65:35, v/v) was the most effective solvent, yielding extracts

with the highest phenolic and flavonoid contents. Species such as *Pinus brutia*, *Quercus robur*, and *Fagus orientalis* showed particularly high phenolic levels and strong antioxidant activities, while *Abies nordmanniana* subsp. *equitrojani* was distinguished by its unique chemical profile and phenolic yield.

GC–MS and HPLC analyses revealed diverse species-specific chemical fingerprints, with several compounds—such as 2-ethylhexanol, methyl stearate, and mono(2-ethylhexyl) adipate—occurring in high concentrations and contributing to the observed bioactivities. Combining chemical profiling with antioxidant, antimicrobial, enzyme-inhibitory, and DNA-protective assays provides a robust framework for linking chemical composition to biological function and identifying species with promising bioactive potential.

These findings highlight the opportunity to valorize forestry byproducts as renewable sources of bioactive compounds, opening avenues for pharmaceutical, nutraceutical, and industrial applications. Future studies should focus on the isolation and structural characterization of key bioactive molecules, in vivo validation of their biological effects, and developing scalable, environmentally friendly extraction processes.

In conclusion, this study demonstrates the potential of bark extracts from seven industrially relevant tree species as renewable sources of bioactive compounds. Future research should focus on the isolation and characterization of key molecules, in vivo validation, scalable green extraction methods, and the influence of ecological factors on species-specific phytochemical diversity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00107-025-02349-2.

Acknowledgements This study was prepared as part of the doctoral dissertation "Determination of Chemical Content, Some Biochemical and Antimicrobial Activities of Some Industrial Tree Bark Extracts." Author contributions Conceptualization: Akça, M., Gür, M., Güder A.; Methodology: Akça, M., Gür, M., Güder A.; Formal analysis and investigation: Akça, M., Gür, M., Güder A.; Writing- original draft preparation: Akça, M.; Writing- review and editing: Akça, M., Gür, M., Güder A. The authors read and approved the final manuscript. Funding: Not applicableData availability: The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.Code availability: Not applicable Declarations Conflict of interest: The authors declare that they have no conflict of interest. Ethical approval: Not applicable. Consent to participate: Not applicable. Consent for publication: All authors gave informed consent to this publication and its content.

Author contributions This manuscript was prepared by MA as part of a doctoral dissertation. MG contributed by drafting the introduction section of the manuscript. AG assisted in the preparation of the materials and methods section. The remaining sections were written by MA. Finally, the entire manuscript was reviewed in its final form by MG and AG.

Funding Not applicable.

Data availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval Not applicable.

Consent for publication All authors gave informed consent to this publication and its content.

Consent to participate Not applicable.

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