



Pyrolysis-based valorization of *Pinus sylvestris* bark waste into bioactive fractions: LC–MS/MS analysis and antioxidant, anti-inflammatory, antidiabetic, and antibacterial activities

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Abstract

In this study, bark waste from the forest industry derived from *Pinus sylvestris* was processed by pyrolysis extraction, and the chemical compositions and biological activities of the resulting light (PLF) and heavy (PHF) fractions were comparatively investigated. When the extraction yields of the pyrolysis fractions were compared, PHF (20.80%) showed a significantly higher recovery than PLF (10.35%). Total phenolic content (TPC) was higher in the PHF (365.80 GAE/g), whereas total flavonoid content (TFC) was concentrated in the PLF (200.80 mg QE/g). LC–MS/MS analyses of the PHF fraction, which exhibited more pronounced biological activity, revealed caffeic acid (7190.65 µg/g) and protocatechuic acid (2694.30 µg/g) as the predominant phenolic compounds. Antioxidant capacity analyses indicated that the PLF exhibited stronger radical scavenging activity, lower IC₅₀ values in DPPH and ABTS^{•+} assays (50.01 and 101.60 µg/mL, respectively) and higher TAC values (901.00 mg TE/L). Fe³⁺ and Cu²⁺ reducing assays demonstrated strong redox activity in both fractions, with Cu²⁺ reducing capacity particularly higher in PLF. In enzyme inhibition studies, the PHF showed stronger inhibition against 5-lipoxygenase (IC₅₀=29.28 µg/mL) and α-glucosidase (IC₅₀=82.50 µg/mL) compared to PLF. The antibacterial potential of the fractions was assessed using *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Klebsiella pneumoniae*. The PHF exhibited significant inhibition zones, particularly against *P. aeruginosa* (17.40 mm) and *S. aureus* (13.40 mm). Consequently, these findings suggest that pyrolysis-based fractionation may be an effective and sustainable method for converting *P. sylvestris* bark waste into functional fractions with high antioxidant, anti-inflammatory, antidiabetic, and antibacterial potential.

Introduction

Plants are important natural sources of bioactive chemicals with diverse biological properties (Chaudhari et al. 2021). In this respect, the identification and recovery of bioactive compounds from plant materials and plant-based wastes are of substantial interest for human health, the food sector and the bioeconomy (Chiocchio et al. 2021). Even though phenolic compounds are commonly associated with fruits and vegetables, woody plant wastes have been identified as valuable and exploitable sources of phenolic-rich fractions (Coşarcă et al., 2019; Tanase et al. 2019). Although these wastes can offer higher economic value than conventional low-value uses and can contribute to sustainability-driven industrial initiatives (Tbatou et al. 2024), the conversion of these residues into high-value products is still rather limited. Wood biomass wastes contain extractable metabolites such as alkaloids, phenolics, pectins, resins and essential oils which, although present in lesser quantities than cellulose, hemicellulose and lignin, may considerably contribute to their biological and functional value (Belt et al. 2017). These compounds are of special relevance for food, pharmaceutical and related applications due to their reported antioxidant, antibacterial and anti-inflammatory activities (Tungmunthum et al. 2018).

Pine species (*Pinus* spp.) comprise approximately 110 taxa worldwide and are of major economic and ecological importance, forming one of the largest forest ecosystems in Europe (Ferreira-Santos et al. 2020a; Saber et al. 2021). Scots pine (*Pinus sylvestris*) is among the most important pine species in Europe because of its broad distribution, ecological adaptability, and economic relevance (Vacek et al. 2016; Brichta et al. 2023). In particular, *P. sylvestris* bark, which is generated in large quantities as a forestry and wood-processing residue, represents a promising biochemical resource compared with other woody by-products (Pals et al. 2022). Pine bark extracts are known to contain phenolic acids, flavonoids, catechin, epicatechin, and taxifolin (Yesil-Celiktas et al. 2009). These compounds are associated with antioxidant and anti-inflammatory effects and may contribute to protection against oxidative stress-related cellular damage and degenerative processes (Saad et al. 2025). In traditional Turkish medicine, the barks of *P. nigra* and *P. sylvestris* have also been used externally for wound healing and in preparations associated with hemorrhoids, diabetes, liver disorders, common cold, bronchitis, abdominal pain, and skin fungal infections (Ari et al. 2017).

The efficient recovery of bioactive compounds from polyphenol-rich by-products is important for both scientific and industrial purposes. These compounds can be obtained using extraction methods based on different physical and chemical principles (Uwineza and Waśkiewicz 2020). However, conventional techniques such as solid–liquid extraction, hydrodistillation, maceration, and Soxhlet extraction often require high solvent and energy consumption, long processing times, and may generate considerable waste (Chemat et al. 2019; Rocha et al. 2018). These limitations have encouraged the development of greener and more economically feasible alternatives (Martins et al. 2023). Among these approaches, pyrolysis is a thermochemical process in which biomass is decomposed under low-oxygen or oxygen-free conditions to produce solid, liquid, and gaseous products (Riekkinen et al. 2022; Freitas et al. 2021; Matos et al. 2021). Compared with solvent-based methods, pyrolysis

can reduce solvent use, processing steps, and solvent residues, making it a potentially more sustainable strategy for biomass valorization (Ferrari et al. 2025). The liquid product, commonly referred to as bio-oil, generally consists of an aqueous light fraction and a denser organic heavy fraction (Bridgwater 2012). Depending on the feedstock and process conditions, these fractions may contain diverse organic compounds, including phenolic constituents associated with antioxidant activity (Gollakota et al. 2023; Wu et al. 2015). Product yield and chemical composition are strongly influenced by parameters such as biomass type, particle size, temperature, and heating rate (Mora et al. 2023; Celeiro et al. 2021).

Oxidative stress, chronic inflammation, antibiotic resistance, and diabetes remain major challenges for public health. Oxidative stress occurs when reactive oxygen species exceed cellular defense capacity, leading to damage in DNA, lipids, and proteins, and is involved in metabolic, neurodegenerative, and cardiovascular disorders (Reddy 2023; Maya-Cano et al. 2021). Chronic inflammation also contributes to the progression of diseases such as metabolic syndrome, atherosclerosis, and certain cancers through persistent immune activation (Chavda et al. 2024; Şeker et al. 2021). In parallel, increasing resistance to commonly used antibiotics has intensified the search for alternative antimicrobial agents from natural sources (Terreni et al. 2021; Murray et al. 2022). In this context, phenolic-rich fractions derived from woody residues, including pine bark, may provide functional value while supporting circular bioeconomy strategies and reducing environmental burdens.

This study focuses on the pyrolysis-based valorization of *P. sylvestris* bark waste through the production of two operationally separated pyrolysis liquid fractions, namely light pyrolysis fraction (PLF) and heavy pyrolysis fraction (PHF), followed by their chemical and biological evaluation. Although pine bark extracts obtained by conventional methods such as maceration, Soxhlet extraction, and solvent-based extraction have been widely investigated (Häsler Gunnarsdottir et al. 2023; Tbatou et al. 2024; Liu et al. 2025), studies on pyrolysis-derived pine bark fractions remain limited. To the best of our knowledge, no previous study has evaluated the liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based phytochemical profile of pyrolysis-derived *P. sylvestris* bark fractions together with their antioxidant, antibacterial, anti-inflammatory, and antidiabetic activities within a single experimental framework. In this context, the present study provides a focused contribution to the sustainable valorization of pine bark waste and highlights its potential as a source of high value-added bioactive fractions for pharmaceutical and industrial biotechnology applications.

Materials and methods

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. Ethanol, methanol, aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), sodium nitrite (NaNO_2), dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), quercetin, Folin–Ciocalteu reagent, neocuproine, 1,1-diphenyl-2-picryl-

hydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), copper(II) chloride (CuCl_2), potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$), gallic acid, butylated hydroxyanisole (BHA), Trolox, trichloroacetic acid (TCA), ascorbic acid, type V soybean lipoxygenase (5-LOX), indomethacin (INDO) and *p*-nitrophenyl- α -D-glucopyranoside (PNP-G) were purchased from Sigma-Aldrich GmbH (Steinheim, Germany). Sulfuric acid (H_2SO_4), sodium phosphate, ammonium molybdate, ferric chloride (FeCl_3), linoleic acid, Mueller–Hinton broth (MHB), Mueller–Hinton agar (MHA), and ampicillin were obtained from Merck KGaA (Darmstadt, Germany). All water was prepared by double distillation using a conventional distillation system.

Plant material

In this study, the plant material consisted of waste bark from *P. sylvestris*. Biomass samples were collected in March 2025 from a forested area located in Sütluçe Village, Elazığ Province (Turkey) (38.593900°N, 38.978770°E). Species identification of the plant material was performed by Prof. Dr. İnanç Özgen from the Department of Bioengineering, Fırat University. The collected biomass samples were first thoroughly washed with deionized water to remove potential physical contaminants and subsequently subjected to air pre-drying. To stabilize the moisture content, the samples were oven-dried at 104 ± 2 °C (Daihan Scientific, ThermoStable IG-105, South Korea). Following drying, the biomass was ground using a mechanical grinder (Waring Blender, 8011ES, USA) to obtain a homogeneous fine powder. This step was carried out to optimize heat transfer and surface area during the pyrolysis processes.

Extraction of waste biomass using a pyrolysis reactor system

The pyrolysis process for obtaining bio-oil from *P. sylvestris* waste bark was performed using a laboratory-scale, PID-controlled fixed-bed pyrolysis reactor, following the method reported by Özgen et al. (2025) with minor modifications. The experimental workflow is depicted schematically in Fig. 1. High-purity nitrogen was used to purge the reactor chamber before heating, and a steady nitrogen flow ensured an inert atmosphere throughout the procedure.

The ground bark biomass (1 kg) was loaded in a quartz sample holder in the reactor and pyrolyzed at 384 ± 2 °C under PID-controlled heating. The volatile compounds formed in the course of thermal decomposition were sent through a high temperature resistant line to a two-stage cooling and condensation system. During condensation and subsequent phase separation, two visually distinct liquid fractions were obtained. The upper/lighter liquid phase was coded as PLF, whereas the lower/denser liquid phase was coded as PHF. These designations were based on the observed phase-separation behavior and were used as operational fraction names, since density, polarity, and molecular-weight distribution were not directly determined in this study. After pyrolysis the liquid phases were separated by a separatory funnel and filtered through Whatman No. 1 filter paper to eliminate remaining solid particles. Each fraction was concentrated under reduced pressure at 60 °C and 250 mbar on a rotary evaporator (Hei-VAP Advantage, Heidolph, Germany). The resulting viscous fractions were

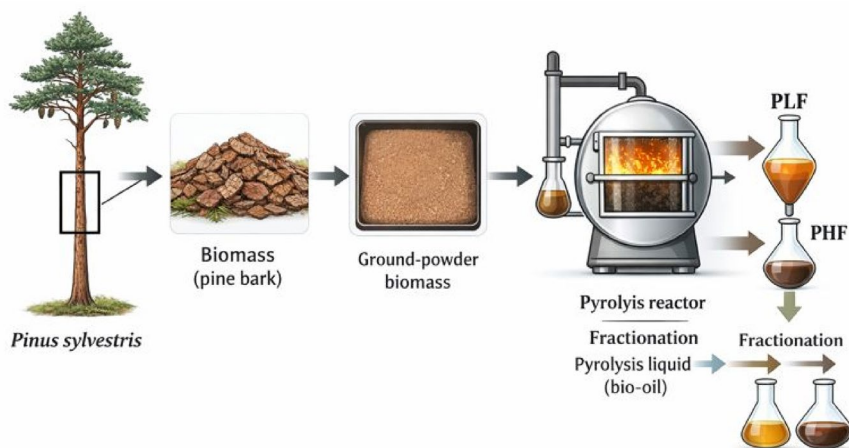


Fig. 1 Schematic representation of biomass extraction using a dual-cooled PID-controlled pyrolysis reactor system

stored in dark glass bottles at 4 °C until further analysis. Extraction yields were calculated using Eq. (1) (Chammam et al. 2024a).

$$Yield (\%) = \frac{m_{DW}}{m_{dm}} \times 100 \quad (1)$$

where m_{DW} represents the mass of the dry extract (g) and m_{dm} denotes the weight of the dry plant material (g).

Determination of total phenolic and flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) of PLF and PHF were determined according to the method reported by Kasangana et al. (2015), with minor modifications. For TPC analysis, 300 μ L of each sample solution (1 mg/mL) was mixed with 3.4 mL deionized water, 0.5 mL methanol, and 200 μ L Folin–Ciocalteu reagent. After vortexing, the mixture was kept at room temperature for 10 min, followed by the addition of 600 μ L sodium Na_2CO_3 (10%, w/v) solution. The reaction mixture was incubated in the dark for 120 min, and the absorbance was measured at 760 nm using a microplate reader (Thermo Multiskan EX, Thermo Scientific, Finland). Deionized water was used instead of the sample for the blank. TPC values were calculated using a gallic acid (GA) calibration curve prepared in the range of 25–250 μ g/mL ($y=0.0056x+0.0102$, $R^2=0.9997$) and expressed as mg gallic acid equivalents per gram of extract (mg GAE/g).

For TFC analysis, 500 μ L of each sample solution (1 mg/mL) was mixed with 3.2 mL methanol (30%, v/v) and 150 μ L NaNO_2 (0.5 M). After 5 min at room temperature, 150 μ L $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (0.3 M) was added, and the mixture was allowed to react for another 5 min. Subsequently, 1.0 mL NaOH (1 M) was added, and the absorbance was recorded at 506 nm. The blank was prepared by replacing the sample with meth-

anol. TFC values were calculated from the quercetin (Q) calibration curve prepared at 25–250 $\mu\text{g/mL}$ ($y=0.0002x-0.0002$, $R^2=0.9993$) and expressed as mg quercetin equivalents per gram of extract (mg QE/g).

LC–MS/MS phytochemical profile analysis

The phytochemical profile of the PHF sample was determined by LC–MS/MS analysis according to Özgen et al. (2025), with minor modifications. The sample solution (20 mg/mL) was filtered through a 0.45 μm syringe filter and transferred directly into LC vials without further pretreatment. Chromatographic separation was performed on an ODS HYPERSIL analytical column (250 $\times$ 4.6 mm, 5 μm) using a Thermo Scientific Dionex Ultimate 3000 UHPLC system coupled to a TSQ Quantum Access Max tandem mass spectrometer. The mobile phase consisted of water containing 0.1% formic acid (A) and methanol (B). The gradient program was as follows: 0–1 min, 100% A; 1–22 min, A decreased to 5%; 22–25 min, 5% A/95% B; and 25–30 min, 100% B, with a total run time of 34 min. The flow rate, column temperature, and injection volume were set to 0.7 mL/min, 30 $^{\circ}\text{C}$, and 20 μL , respectively. Mass spectrometric detection was carried out using an electrospray ionization source in both positive and negative ion modes. Quantification was performed in multiple reaction monitoring (MRM) mode using optimized precursor/product ion transitions and collision energies for each analyte (Yılmaz 2020). The source conditions were maintained as follows: capillary temperature, 300 $^{\circ}\text{C}$; vaporizer temperature, 350 $^{\circ}\text{C}$; sheath gas, 30 Arb; auxiliary gas, 13 Arb; spray voltage, 4000 V in positive mode and 2500 V in negative mode; and discharge current, 4.0 μA . Data were processed using Xcalibur 2.2 software. Calibration curves were constructed using analytical reference standards of the phenolic compounds listed in Table 5, and the results were expressed as $\mu\text{g/g}$ extract.

Determination of total antioxidant capacity (TAC)

The TAC of the samples was determined using a spectrophotometric method based on the phosphomolybdenum complex (Prieto, 1999). Briefly, 500 μL of each sample was mixed with 2500 μL of deionized water and 1000 μL of reagent solution (0.6 M H_2SO_4 , 28 mM sodium phosphate, and 4 mM ammonium molybdate) in individual tubes. The reaction mixtures were incubated at 95 $^{\circ}\text{C}$ for 90 min and subsequently cooled to room temperature. The absorbance of the resulting complexes was measured at 695 nm using a microplate reader against a blank containing 500 μL of deionized water. The same procedure was applied to standard antioxidant solutions prepared within the concentration range of 10–250 $\mu\text{g/mL}$. TAC values were calculated using calibration curves constructed with Trolox (T) ($y=0.0018x+0.0116$, $R^2=0.9987$) and BHA ($y=0.0027x-0.0036$, $R^2=0.9989$) standards and expressed as mg TE/L and mg BHAE/L, respectively.

Antioxidant capacity assays

The antioxidant capacity of the test samples was evaluated using DPPH and ABTS^{•+} radical scavenging assays. The DPPH free radical scavenging activity of the samples was evaluated using a modified version of the method reported by Yildirim and Gül (2025), adapted to the experimental conditions. Accordingly, the samples were initially prepared in DMSO at a concentration of 5 mg/mL and subsequently serially diluted to obtain concentrations of 0.25, 0.063, 0.016, and 0.004 mg/mL. An aliquot of 10 µL from each dilution was mixed with 1.90 mL of freshly prepared methanolic DPPH solution (0.1 mM). The blank was prepared in parallel by mixing 10 µL of the solvent with 1.90 mL of the methanolic DPPH solution. The reaction mixtures were incubated in the dark for 30 min, after which the absorbance values were measured at 517 nm using a microplate reader. The percentage inhibition of DPPH free radicals was calculated according to Eq. 2, and the mean effective concentration (IC₅₀), defined as the concentration required to scavenge 50% of the DPPH free radicals, was determined. Ascorbic acid (AA) was used as the positive control.

$$\text{DPPH free radical scavenging activity (\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \quad (2)$$

where A_0 represents the absorbance of the control sample containing only the reagents without the test sample, and A_1 denotes the absorbance of the tested samples.

The ABTS^{•+} radical scavenging capacity of the samples was determined based on an experimentally adapted version of the method reported by Zou et al. (2011). Briefly, a 7 mM ABTS solution was mixed with 2.45 mM K₂S₂O₈, and the mixture was incubated in the dark at room temperature for 16 h to allow complete radical generation. The resulting ABTS^{•+} stock solution was diluted with ethanol to obtain an absorbance of 0.700 ± 0.050 at 734 nm. Sample solutions were prepared in DMSO at concentrations of 5 mg/mL, 0.25, 0.063, 0.016, and 0.004 mg/mL. Aliquots of 10 µL from each concentration were transferred into microplate wells, followed by the addition of 190 µL of ABTS^{•+} solution. After incubation at room temperature for 30 min, absorbance values were measured at 734 nm using a microplate reader. The percentage inhibition of ABTS^{•+} radicals was calculated according to Eq. 2 as described for the DPPH assay.

Antioxidant activity analysis based on the reducing capacity of Fe³⁺ and Cu²⁺

The antioxidant activity of the test samples was also evaluated based on their Fe³⁺ and Cu²⁺ reducing capacities. The Fe³⁺-to-Fe²⁺ reducing capacities of the samples were determined according to the electron transfer-based method described by Menevseoglu et al. (2025). Briefly, 500 µL of each sample solution was mixed with equal volumes of 0.2 M phosphate buffer (pH 6.6) and 1% (w/v) K₃[Fe(CN)₆], and the reaction mixture was incubated at 50 °C for 20 min. Following incubation, the reaction was terminated by adding 500 µL of 10% (w/v) TCA, and the mixture was centrifuged at 5000 rpm for 5 min. An aliquot of 500 µL of the resulting supernatant was then combined with 500 µL of 0.1% (w/v) FeCl₃ solution. After standing at

room temperature for 10 min, the absorbance of the reaction mixture was measured at 700 nm against a blank containing deionized water only, using a microplate reader. T (10–250 $\mu\text{g/mL}$) and BHA (10–100 $\mu\text{g/mL}$) standards were prepared following the same procedure, and calibration curves were obtained with regression equations of $y=0.0087x+0.0283$ ($R^2=0.9979$) and $y=0.0325x+0.0798$ ($R^2=0.9961$), respectively. The Fe^{3+} reducing capacities of the samples were calculated using these calibration curves and expressed as mg TE/L and mg BHAE/L.

The ability of the samples to reduce Cu^{2+} to the Cu^+ form was determined using an electron transfer–based method as described by Apak et al. (2006). Accordingly, 1.0 mL of 0.01 M CuCl_2 , 7.5 mM neocuproine solution and 1.0 M ammonium acetate buffer (pH 7.0) were added to 0.5 mL of the sample solution and the total content was adjusted to 4.0 mL with deionized water. The reaction mixture was maintained at room temperature in the dark for 30 min. After incubation, absorbance values were read at 450 nm using a microplate reader against a blank of an equal amount of deionized water in place of the sample. The same procedure was applied to T and BHA standard antioxidant solutions prepared in the concentration range of 10–250 $\mu\text{g/mL}$, yielding calibration equations of $y=0.0009x+1.3067$ ($R^2=0.9944$) and $y=0.009x+1.3112$ ($R^2=0.9974$), respectively. The Cu^{2+} reducing capacities of the samples were calculated using these calibration curves and expressed as mg TE/L and mg BHAE/L.

Determination of anti-inflammatory activity

The anti-inflammatory activity assay was carried out to determine the inhibitory activity of the samples against 5-LOX in the 96-well microplate format according to the protocols of Phosrithong and Nuchtavorn (2016) and Yıldırım et al. (2019) with slight modifications according to the experimental conditions. In the test, 10 μL of the sample or reference inhibitor at different concentrations were sequentially added to 20 μL of ethanol, deionized water and sodium borate buffer (0.1 M, pH 9.0). Then 25 μL of the solution of 5-LOX (20,000 U/mL) was added and the mixture was incubated at 25 °C for 5 min. The reaction was started by adding 100 μL of 0.6 mM linoleic acid solution to the microplate. The microplate was gently shaken. The 5-LOX activity was determined by monitoring the increase in absorbance at 234 nm, corresponding to the oxidation of linoleic acid. The increase was recorded in kinetic mode for 6 min. The percentage of 5-LOX inhibition was determined using Eq. 2 with INDO as the positive control.

Determination of antidiabetic (α -glucosidase inhibitory) activity

The antidiabetic potential of the samples based on α -glucosidase inhibition was evaluated using a modified version of the PNP-G method reported by Ben Khadher et al. (2023). Briefly, 10 μL of the samples at different concentrations were mixed with 40 μL of sodium phosphate buffer (0.1 M, pH 6.9) and 50 μL of α -glucosidase enzyme solution (1 U/mL, derived from *Saccharomyces cerevisiae*), followed by pre-incubation at 25 °C for 15 min. Then the enzymatic reaction was started by adding 50 μL of 5 mM PNP-G solution prepared in the same buffer. The reaction mixtures were then incubated for an additional 15 min at 25 °C. The amount of p-nitrophenol produced

by enzymatic hydrolysis was evaluated by measuring the absorbance at 405 nm using a microplate reader. Acarbose was employed as a positive control and percentage of α -glucosidase inhibition was estimated using Eq. 2.

Antibacterial studies

Microorganisms employed for antibacterial assessments

In this study, clinically relevant standard reference bacterial strains were used for antibacterial activity assays. The test organisms included *Escherichia coli* ATCC 25,922, *Pseudomonas aeruginosa* ATCC 27,853, *Enterococcus faecalis* ATCC 29,212, *Staphylococcus aureus* ATCC 25,923, *Salmonella typhimurium* ATCC 23,566, and *Klebsiella pneumoniae* ATCC 13,883. All strains were obtained from standard ATCC stock cultures maintained in the Microbiology Laboratory of the Department of Genetics and Bioengineering, Gumushane University.

Determination of antibacterial activity

Antibacterial activity was investigated by the agar well diffusion method as described by Karpuz et al. (2025) with minor adjustment on the experimental conditions. The bacterial strains were cultivated in MHB for 16–18 h to reach the logarithmic phase of growth prior to the experiment. Aliquots of 100 μ L from each active culture were equally spread on the surface of MHA (Oxoid CM337, Basingstoke, Hampshire, UK) with sterile cotton swabs following the diffusion-based standard methods proposed by the Clinical and Laboratory Standards Institute (CLSI). After inoculation the plates were allowed to stand at room temperature for 15–20 min to allow the bacteria to attach to the surface of the agar. Subsequently, wells of 6 mm diameter were aseptically punched into the agar using a sterile borer. Sample solutions prepared in deionized water at different concentrations (10, 5, 1, and 0.5 mg/mL) were applied to each well at a volume of 50 μ L. The inoculation plates were incubated at 37 °C for 18–24 h. Ampicillin (25 μ g/mL) was employed as the positive control. After incubation, the antibacterial activity was determined by measuring the widths of the inhibitory zones in millimeters (mm). All experimental procedures and evaluations were conducted in accordance with CLSI (2020) guidelines (Wayne 2020).

Data analysis

All experiments were performed independently in triplicate. The results are expressed as mean $\pm$ standard deviation (SD). Statistical analyses were conducted using GraphPad Prism software version 8.0 (GraphPad Software Inc., La Jolla, CA, USA). To evaluate differences among data groups, one-way analysis of variance (one-way ANOVA) was performed using IBM SPSS Statistics version 21. When significant differences were detected by ANOVA, Tukey's multiple comparison test was applied to determine differences between group means. A p-value < 0.05 was considered statistically significant.

Results and discussion

Comparative analysis of extraction yields from pyrolysis products

The extraction yields of the pyrolysis-derived light and heavy fractions obtained from *P. sylvestris* bark waste were determined as 10.35% for PLF and 20.80% for PHF. Thus, PHF showed an approximately two-fold higher yield than PLF, indicating that the heavy fraction was the primary recoverable liquid product under the applied pyrolysis conditions. This could be connected to the accumulation of less volatile and larger molecular weight compounds generated during the thermal degradation of lignocellulosic bark matrix. On the other hand, the lower yield of PLF may be attributed to the higher volatility of low molecular weight molecules and their partial loss or restricted recovery during condensation. Thus, the higher extraction efficiency of PHF than that of PLF could be attributed to its larger molecular weight and phenolic-rich composition. Similar observations were reported by Schmitt et al. (2018).

The extraction yields obtained in this study are comparable to, and in some cases higher than, those reported for different *Pinus* species using conventional or assisted extraction techniques. Chamam et al. (2024b) reported extraction yields ranging from 0.50% to 12.52% for cone fractions of *P. halepensis*, *P. brutia*, and *P. pinea* using hexane, ethyl acetate, and methanol. In another study, aqueous extracts from cone fractions of the same species showed yields between 1.01% and 3.93% (Chamam et al. 2024c). Mohareb et al. (2017) reported acetone extraction yields of 2.39–3.75% for needles and 3.74–6.61% for bark samples of *P. halepensis*. For *P. sylvestris* bark, Pals et al. (2022) obtained yields of 11.4–14.5% using accelerated solvent extraction and 12.6–19.9% using microwave-assisted extraction with water at 70–110 °C, while higher temperatures increased the yields to 25.8–29.1%. Similarly, Erol et al. (2025) reported extraction yields of 7.03–15.03% for *P. nigra* bark extracts under different ultrasound-assisted extraction conditions.

When these literature data are considered, the PLF yield obtained in the present study falls within the range reported for several assisted extraction systems, whereas the PHF yield appears particularly notable. This result indicates that pyrolysis may provide an efficient alternative for recovering liquid fractions from *P. sylvestris* bark waste, especially in terms of heavy fraction formation. Differences among studies can be attributed to the biomass source, plant fraction, particle size, extraction or pyrolysis conditions, and process parameters such as temperature, pressure, and residence time, which are known to affect both product yield and bio-oil characteristics (Rover et al. 2014; Marathe et al. 2019, 2020). Overall, the yield data support the suitability of pyrolysis-based fractionation as a promising approach for the valorization of *P. sylvestris* bark waste.

Determination of TAC, TPC, and TFC profiles

The determination of TPC, TFC, and TAC is commonly used to evaluate the phytochemical richness and antioxidant potential of plant-derived extracts (Lee et al. 2018; Amarowicz and Pegg 2019). Phenolic compounds and flavonoids are among the major antioxidant phytochemicals in plants, and higher TPC and TFC values

are often associated with stronger antioxidant responses (Regmi and Sharma 2023). However, TAC reflects the cumulative contribution of different antioxidant components, including phenolics, flavonoids, vitamins, and other redox-active compounds, as well as their possible synergistic interactions (Silvestrini et al. 2023). In the present study, the TPC, TFC, and TAC values of PLF and PHF were determined, and the results are presented in Table 1.

The results revealed clear differences between the two pyrolysis-derived fractions. PHF showed a higher TPC value (365.80 ± 2.32 mg GAE/g) than PLF (348.12 ± 4.47 mg GAE/g), suggesting that phenolic-rich and relatively higher molecular weight compounds were more concentrated in the heavy phase. This may be related to the accumulation of lignin-derived phenolic constituents during pyrolysis. In contrast, PLF exhibited a higher TFC value (200.8 ± 3.61 mg QE/g) than PHF (157.47 ± 2.52 mg QE/g), which may be associated with the preferential transfer of relatively lower molecular weight flavonoid-like compounds into the light fraction during pyrolysis and condensation. Therefore, the differences in TPC and TFC appear to reflect the fraction-dependent distribution of phenolic compounds according to their polarity, molecular size, and condensation behavior (Ko et al. 2014). Similar compositional differences have been reported for heavy phases obtained from the pyrolysis of pine bark and other lignocellulosic biomass, which are often enriched in lignin degradation products such as phenolic acids, guaiacol, and syringol derivatives (Kanaujia et al. 2014).

The TAC results further supported the chemical distinction between PLF and PHF. When expressed as T equivalents, PLF showed a higher antioxidant capacity (901.00 ± 7.91 mg TE/L) than PHF (706.56 ± 5.72 mg TE/L). A similar pattern was observed for BHA equivalents, with values of 611.93 ± 4.27 mg BHA/E/L for PLF and 482.30 ± 3.48 mg BHA/E/L for PHF. These findings indicate that the antioxidant capacity of the fractions was not determined solely by TPC. Instead, the structural features of antioxidant molecules, hydroxyl group positions, electron-donating capacity, and possible interactions among compounds may also influence the overall antioxidant response (Apak et al. 2016). In this respect, the higher TAC of PLF, despite its lower TPC, is consistent with its higher TFC and with the known contribution of flavonoid-type compounds to antioxidant activity (Apak et al. 2016; Prior et al. 2005).

To the best of our knowledge, no previous study has systematically evaluated the TPC, TFC, and TAC profiles of PLF and PHF obtained from *P. sylvestris* bark by

Table 1 Quantitative assessment of antioxidant capacity phenolic and flavonoid content*

Samples	TAC		TPC (mg GAE/ g)	TFC (mg QE/g)
	Trolox (mg TE/L)	BHA (mg BHA/E/L)		
PLF	901.00 ± 7.91^a	611.93 ± 4.27^a	348.12 ± 4.47^b	200.80 ± 3.61^a
PHF	706.56 ± 5.72^b	482.30 ± 3.48^b	365.80 ± 2.32^a	157.47 ± 2.52^b

*TAC total antioxidant capacity, TPC total phenolic content, TFC total flavonoid content, TE Trolox equivalent, BHA/E butylated hydroxyanisole equivalent, GAE gallic acid equivalent, QE quercetin equivalent, PLF pyrolysis light fraction, PHF pyrolysis heavy fraction.

All values are presented as mean $\pm$ SD ($n=3$). Different superscript letters within the same column indicate statistically significant differences according to Tukey's multiple comparison test assuming equal variances ($p < 0.05$)

pyrolysis. However, several studies have reported TPC and TFC contents of *P. sylvestris* extracts obtained using conventional or assisted extraction techniques. Nisca et al. (2021) reported TPC values of 18.46–58.41 mg GAE/g for *P. sylvestris* bark extracts obtained by ethanol-based ultrasonic-assisted extraction (UAE) and microwave-assisted single-cycle aqueous extraction (MAE). Skrypnik et al. (2019) found TPC and TFC values of 4.40–12.33 mg GAE/g and 2.11–5.20 mg QE/g, respectively, in aqueous bark extracts from the Kaliningrad region. Similarly, Dziedzinski et al. (2020) reported TPC and TFC values of 0.86 mg GAE/g and 8.30 mg QE/g for aqueous extracts of *P. sylvestris* shoots, while Hamad et al. (2019) reported 88 mg GAE/g and 22 mg catechin equivalents/g for methanol–water extracts of industrially used *P. sylvestris* bark from Türkiye. Topal (2020) also reported the highest TPC and TFC values as 131.82 mg GAE/g and 14.44 mg QE/g, respectively, in ethanol extracts of *P. sylvestris* cones collected from Gümüşhane.

Compared with these conventionally prepared extracts, both PLF and PHF showed markedly higher TPC and TFC values. This suggests that pyrolysis-based fractionation may enhance the recovery or formation of phenolic constituents from *P. sylvestris* bark. The elevated values observed in this study may be associated not only with extraction efficiency but also with pyrolysis-induced transformation of lignocellulosic structures into lower molecular weight phenolic derivatives. Nevertheless, this interpretation should be considered together with the targeted phytochemical profile obtained by LC–MS/MS analysis. Overall, the high TPC, TFC, and TAC values indicate that PLF and PHF are phenolic-rich fractions with notable antioxidant potential, supporting the use of pyrolysis as a promising approach for the valorization of *P. sylvestris* bark waste.

Evaluation of the antioxidant activities

DPPH and ABTS•⁺ radical scavenging assays are widely used to evaluate the antioxidant activity of bark-derived extracts because they provide complementary information on radical neutralization in different chemical environments. While the DPPH assay is particularly responsive to lipophilic antioxidants, the ABTS•⁺ assay allows the evaluation of both hydrophilic and lipophilic antioxidant compounds (Häsler Gunnarsdottir et al. 2023; Ferreira-Santos et al. 2020b). Therefore, both assays were used in the present study to compare the radical scavenging capacities of PLF and PHF.

The antioxidant activities of PLF and PHF were evaluated based on inhibition percentages and IC₅₀ values calculated from dose–response curves, and the results are summarized in Table 2. In both assays, lower IC₅₀ values indicate stronger radical scavenging activity. PLF and PHF showed DPPH inhibition values of 69.68% and 68.19%, respectively, whereas markedly higher inhibition was observed in the ABTS•⁺ assay, with values of 98.15% for PLF and 96.84% for PHF. The stronger response in the ABTS•⁺ assay suggests that the fractions contain antioxidant compounds that are particularly effective in electron transfer-based radical scavenging systems.

The IC₅₀ values further confirmed the higher antioxidant performance of PLF. In the DPPH assay, PLF exhibited a lower IC₅₀ value (50.01 µg/mL) than PHF

Table 2 Antioxidant activity of PLF and PHF fractions determined by DPPH and ABTS^{•+} radical scavenging assays *

Samples and standard	DPPH inhibition (%)	ABTS ^{•+} inhibition (%)	DPPH ABTS ^{•+} IC ₅₀ (μg/mL)		RP (DPPH)	RP (ABTS ^{•+})
PLF	69.68 ± 0.12 ^a	98.15 ± 0.12 ^a	50.01 ± 0.60 ^a	101.60 ± 1.80 ^b	0.98	1.72
PHF	68.19 ± 0.06 ^b	96.84 ± 0.02 ^b	54.28 ± 1.01 ^b	132.40 ± 0.60 ^c	1.07	2.24
AA	—	—	50.93 ± 1.02 ^a	59.01 ± 0.490 ^a	—	—

*DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS^{•+}, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation; IC₅₀, half-maximal inhibitory concentration; PLF, pyrolysis light fraction; PHF, pyrolysis heavy fraction; RP, relative potency, calculated according to Eq. (S1); AA, ascorbic acid. All values are presented as mean ± SD (*n* = 3). Different superscript letters within the same column indicate statistically significant differences according to Tukey's multiple comparison test assuming equal variances (*p* < 0.05)

(54.28 μg/mL), indicating slightly stronger radical scavenging efficiency. PLF also showed a lower IC₅₀ value in the ABTS^{•+} assay, supporting its higher antioxidant activity across both test systems. This difference is consistent with the higher TAC and TFC values previously determined for PLF. Although PHF had a higher TPC, the antioxidant response was not governed solely by total phenolic content; the molecular structure of antioxidant compounds, hydroxyl group positions, electron-donating capacity, and interactions among constituents may also affect radical scavenging efficiency (Apak et al. 2016). Accordingly, the higher activity of PLF may be related to its flavonoid-rich profile and the presence of lower molecular weight redox-active compounds, which are known to contribute strongly to antioxidant assays such as DPPH, ABTS^{•+}, and CUPRAC (Cupric Reducing Antioxidant Capacity) (Apak et al. 2016; Prior et al. 2005; Niroula et al. 2019; Ferreira-Santos et al. 2020b).

AA was used as the positive control and showed strong activity in the DPPH assay, with an IC₅₀ value of 50.93 μg/mL. Notably, the DPPH IC₅₀ value of PLF (50.01 μg/mL) was close to that of AA under the same experimental conditions. This finding indicates that the pyrolysis-derived PLF possesses considerable antiradical activity and may represent a promising natural antioxidant fraction obtained from *P. sylvestris* bark waste. However, this comparison should be interpreted within the applied assay conditions, since antioxidant values can vary depending on biomass source, extraction procedure, reaction medium, incubation time, and reference antioxidant (Drózdż and Pyrzynska 2021; Apak et al. 2013).

Based on current knowledge, the DPPH and ABTS^{•+} radical scavenging activities of PLF and PHF derived from *P. sylvestris* bark by pyrolysis have not been previously documented. However, several studies have evaluated antioxidant profiles of *Pinus* bark extracts obtained using conventional or assisted extraction techniques. Nisca et al. (2021) reported that the antioxidant activity of *P. sylvestris* bark extracts strongly depended on extraction method and solvent system; DPPH activity reached 74.59% using ethanol-based UAE but decreased to approximately 40% with MAE, while ABTS^{•+} activity was highest with UAE (80.36%) and around 60% with MAE. Compared with these values, PLF showed comparable DPPH inhibition (69.68%) and a higher ABTS^{•+} response (98.15%), suggesting that pyrolysis-based fractionation can generate antioxidant-rich fractions with strong radical scavenging capacity.

The present results are also comparable with those reported for other pine bark extracts. Tbatou et al. (2024) reported a DPPH IC₅₀ value of 9.76 μg/mL for *P. pin-*

aster bark extract obtained by maceration using 70:30 (v/v) ethanol/water, while AA showed an IC_{50} value of $5.59 \pm 0.03 \mu\text{g/mL}$ under their experimental conditions. In the present study, different assay conditions resulted in an AA IC_{50} value of $50.93 \mu\text{g/mL}$, and the PLF and PHF values were close to this positive control. Similarly, Ferreira-Santos et al. (2020b) reported that 50% ethanol extracts of *P. pinaster* bark showed IC_{50} values of $49.74 \mu\text{g/mL}$ for DPPH and $59.41 \mu\text{g/mL}$ for ABTS $^{\bullet+}$, whereas 90% ethanol extracts showed lower activity, with IC_{50} values of 100.10 and 112.10 $\mu\text{g/mL}$, respectively. In this context, the IC_{50} values obtained for PLF and PHF fall within a relevant range compared with active pine bark extracts reported in the literature.

To support a more standardized comparison, relative potency (RP) was also calculated using the sample IC_{50} /positive control IC_{50} ratio according to Eq. (S1), as proposed by Ancuceanu et al. (2024). The RP values, added to Table 2, confirmed the antioxidant ranking obtained from IC_{50} results and showed that PLF had stronger activity than PHF, particularly in the DPPH assay. Overall, these findings indicate that pyrolysis-derived fractions from *P. sylvestris* bark, especially PLF, possess considerable antioxidant potential. The detailed RP-based classification is provided in the Supplementary Information (Table S1).

Evaluation of the Fe^{3+} and Cu^{2+} reducing capacities

The Fe^{3+} and Cu^{2+} reducing assays were used to evaluate the electron-donating capacity of PLF and PHF. As shown in Table 3, both fractions exhibited strong reducing activity in both assay systems. In the Fe^{3+} reducing assay, PLF and PHF showed very similar values, with 1714.20 ± 17.93 and $1714.48 \pm 9.30 \text{ mg TE/L}$, respectively. A comparable trend was also observed when the results were expressed as BHA equivalents, with values of 450.95 ± 4.80 and $451.03 \pm 2.49 \text{ mg BHAE/L}$ for PLF and PHF, respectively. These results indicate that both pyrolysis-derived fractions possess a strong and nearly equivalent Fe^{3+} reducing capacity, suggesting the presence of electron-donating compounds in both fractions. This behavior is consistent with the known redox reactivity of phenolic compounds (Csepregi et al. 2016).

In the Cu^{2+} reducing assay, PLF showed a higher reducing capacity than PHF. The values were determined as $9711.07 \pm 16.97 \text{ mg TE/L}$ for PLF and $6548.11 \pm 19.25 \text{ mg TE/L}$ for PHF. When expressed as BHA equivalents, the corresponding values were 970.61 ± 1.70 and $654.31 \pm 1.92 \text{ mg BHAE/L}$, respectively. This difference indicates that PLF had a greater ability to reduce Cu^{2+} to Cu^{+} , suggesting stronger electron-transfer efficiency in the CUPRAC system. Since the Cu^{2+} reducing assay is respon-

Table 3 Fe^{3+} and Cu^{2+} reduction capacities of PLF and PHF fractions *

Samples	Fe^{3+} reduction		Cu^{2+} reduction	
	Trolox (mg TE/L)	BHA (mg BHAE/L)	Trolox (mg TE/L)	BHA (mg BHAE/L)
PLF	1714.20 ± 17.93^a	450.95 ± 4.80^a	9711.07 ± 16.97^a	970.61 ± 1.70^a
PHF	1714.48 ± 9.30^a	451.03 ± 2.49^a	6548.11 ± 19.25^b	654.31 ± 1.92^b

*TE Trolox equivalent, BHAE butylated hydroxyanisole equivalent, PLF pyrolysis light fraction, PHF pyrolysis heavy fraction

All values are presented as mean $\pm$ SD ($n=3$). Different superscript letters within the same column indicate statistically significant differences according to Tukey's multiple comparison test assuming equal variances ($p < 0.05$)

sive to both hydrophilic and lipophilic antioxidants and is often associated with phenolic and flavonoid contents, the higher Cu^{2+} reducing capacity of PLF is consistent with its higher TFC and TAC values (Munteanu and Apetrei 2021; Apak et al. 2008). Overall, the Fe^{3+} and Cu^{2+} reducing results show that PLF and PHF contribute to antioxidant activity not only through radical scavenging but also through electron-donating mechanisms. While both fractions displayed almost identical Fe^{3+} reducing capacities, PLF was more effective in the Cu^{2+} reducing system. This suggests that the two fractions may differ in the type and reactivity of their redox-active constituents, rather than only in their total phenolic levels.

To the best of our knowledge, the simultaneous evaluation of Fe^{3+} and Cu^{2+} reducing capacities of PLF and PHF obtained from *P. sylvestris* bark by pyrolysis has not been previously reported. However, reducing capacities of *Pinus* bark extracts obtained by different extraction methods have been investigated. Şeker et al. (2021) reported an Fe^{3+} reducing capacity of 70 μM TE for *P. sylvestris* bark extracts prepared by a multistep solvent-based extraction procedure. In the present study, PLF and PHF showed Fe^{3+} reducing values of 1714.20 and 1714.48 mg TE/L, respectively. Although direct comparison is limited due to differences in extraction procedures and expression units, the present results indicate that pyrolysis-derived fractions can retain or generate compounds with strong Fe^{3+} reducing potential. Similarly, Drózdź and Pyrzyńska (2021) reported strong Cu^{2+} reducing capacity for *P. sylvestris* bark extracts obtained using a 60% ethanol–water mixture. In the present study, PLF and PHF also showed high Cu^{2+} reducing capacities, particularly PLF. However, because the literature data are expressed on a different basis, direct numerical comparison should be interpreted cautiously. Erol et al. (2025) also reported notable Fe^{3+} reducing capacities for *P. nigra* bark extracts obtained by UAE, depending on extraction conditions. Together, these studies indicate that pine bark is a rich source of redox-active compounds, while the present findings suggest that pyrolysis-based fractionation can provide an alternative route for obtaining antioxidant fractions from *P. sylvestris* bark.

Taken together, the reducing assays support the antioxidant potential of both PLF and PHF. The high Fe^{3+} reducing values of both fractions and the stronger Cu^{2+} reducing capacity of PLF indicate that pyrolysis-derived fractions contain functionally active electron-donating compounds. These findings further support the potential of *P. sylvestris* bark waste as a source of natural antioxidant fractions, while additional compositional and mechanistic studies would be useful to clarify the specific compounds responsible for the observed redox activity.

Evaluation of the anti-inflammatory and antidiabetic activities

Inflammation and postprandial hyperglycemia are important targets in the search for natural bioactive compounds. In this context, 5-LOX inhibition is commonly used to assess anti-inflammatory potential because of its role in leukotriene biosynthesis, whereas α -glucosidase inhibition is relevant for delaying carbohydrate digestion and reducing postprandial glucose absorption (Hussain et al. 2020; Kashtoh and Baek 2022). Plant-derived phenolics and related natural products have been widely investigated for these activities, with INDO and acarbose commonly used as reference

inhibitors for anti-inflammatory and antidiabetic assays, respectively (William et al. 2019; Kim et al. 2022; Chammam et al. 2024c).

The 5-LOX inhibitory activities of PLF and PHF were evaluated based on IC_{50} values, as shown in Table 4. PHF exhibited a markedly stronger inhibitory effect than PLF under the applied experimental conditions, indicating a higher anti-inflammatory potential. This result suggests that the anti-inflammatory response was not governed only by TFC or TAC values. Although PLF showed higher TFC and TAC, PHF displayed stronger 5-LOX inhibition, which may be associated with the higher TPC of PHF and the presence of more structurally complex phenolic or aromatic constituents formed during pyrolysis. Since 5-LOX inhibition may depend on the structural properties of individual compounds rather than total antioxidant capacity alone, the stronger activity of PHF appears to reflect its distinct chemical composition (Yildirim et al. 2024; Metkin et al. 2025).

INDO, used as the positive control, showed the strongest inhibitory activity, as expected. Nevertheless, PHF exhibited notable activity within a natural multicomponent matrix, with an IC_{50} value of 29.28 $\mu\text{g/mL}$. Since this activity was obtained from a pyrolysis-derived fraction rather than a purified compound, the result indicates that PHF may contain constituents with relevant 5-LOX inhibitory potential. However, further compound-specific and mechanistic studies would be required to identify the active molecules responsible for this effect.

To the best of our knowledge, no previous study has directly evaluated the 5-LOX inhibitory activities of PLF and PHF obtained from *P. sylvestris* bark by pyrolysis. However, limited studies have investigated anti-inflammatory effects of *P. sylvestris* extracts prepared by conventional methods. Vigo et al. (2005) evaluated a *P. sylvestris*-derived extract in a cell-based inflammatory model and reported only a limited nitric oxide (NO)-scavenging effect at the highest tested concentration. Compared with that report, the lower IC_{50} value observed for PHF in the present 5-LOX assay suggests that pyrolysis-derived bark fractions may provide a more active anti-inflammatory fraction under the applied in vitro conditions. This difference may be related to the distinct chemical composition generated during pyrolysis, although direct mechanistic confirmation was not performed in this study.

The antidiabetic potential of PLF and PHF was assessed through α -glucosidase inhibition, using acarbose as the positive control. As presented in Table 4, PLF and PHF showed IC_{50} values of 92.01 $\mu\text{g/mL}$ and 82.50 $\mu\text{g/mL}$, respectively, while acar-

Table 4 Inhibitory activities of PLF and PHF fractions on 5-LOX and α -glucosidase *

Samples and standards**	5-LOX inhibition activity	α -glucosidase inhibition activity
	IC_{50} ($\mu\text{g/mL}$)	
PLF	90.05 $\pm$ 0.23 ^c	92.01 $\pm$ 2.00 ^a
PHF	29.28 $\pm$ 0.42 ^b	82.5 $\pm$ 1.10 ^b
INDO	4.43 $\pm$ 0.02 ^a	—
Acarbose	—	91.22 $\pm$ 0.25 ^a

*5-LOX, 5-lipoxygenase; IC_{50} , half-maximal inhibitory concentration; INDO, indomethacin; PLF, pyrolysis light fraction; PHF, pyrolysis heavy fraction. All values are presented as mean $\pm$ SD ($n=3$). Different superscript letters within the same column indicate statistically significant differences according to Tukey's multiple comparison test assuming equal variances ($p<0.05$)

** INDO and Acarbose were used as positive controls

bose exhibited an IC_{50} value of $91.22 \pm 0.25 \mu\text{g/mL}$ under the same assay conditions. These results indicate that PHF showed slightly stronger α -glucosidase inhibition than acarbose, whereas PLF displayed comparable activity. The stronger activity of PHF may be related to its higher phenolic content and the possible contribution of aromatic, resinous, or lignin-derived compounds concentrated in the heavy fraction. Such compounds may interact with α -glucosidase through hydrophobic interactions and π - π stacking with aromatic residues in the enzyme active site, as reported for phenolic and aromatic inhibitors (Li et al. 2021; Riyaphan et al. 2021; Aleixandre et al. 2022; Liu et al. 2023).

Although no study has directly reported the α -glucosidase inhibitory activities of pyrolysis-derived PLF and PHF from *P. sylvestris* bark, several studies have evaluated related activities in different *Pinus* species. Dakhlaoui et al. (2023) reported α -glucosidase inhibitory activity for essential oils from *P. halepensis* needles, with IC_{50} values ranging from 254.45 to 529.53 $\mu\text{g/mL}$. Compared with these values, both PLF and PHF showed lower IC_{50} values, indicating stronger inhibitory activity under their respective assay conditions. Chamam et al. (2024c) reported that only the aqueous leaf extract of *P. halepensis* showed pronounced α -glucosidase inhibition among the tested extracts of *P. halepensis*, *P. brutia*, and *P. pinea*, with an IC_{50} value of 24.70 $\mu\text{g/mL}$, whereas acarbose showed 4.84 $\mu\text{g/mL}$ in the same study. In the present work, PHF and PLF showed activities close to acarbose under the applied methodology, suggesting that pyrolysis-based fractionation may reveal inhibitory potential in *P. sylvestris* bark. Similarly, Erol et al. (2025) reported an IC_{50} value of 380 $\mu\text{g/mL}$ for α -glucosidase inhibition in *P. nigra* bark extracts obtained by optimized UAE, while acarbose showed 720 $\mu\text{g/mL}$ under the same conditions. Although direct numerical comparison is limited by differences in species, extraction procedures, assay conditions, and reference inhibitor responses, these studies collectively indicate that pine bark is a promising source of α -glucosidase inhibitory compounds. The present findings further suggest that pyrolysis-derived fractions, especially PHF, may offer an alternative route for obtaining bark-derived bioactive fractions without conventional solvent-based extraction.

Overall, PLF exhibited stronger general antioxidant capacity, whereas PHF showed more pronounced 5-LOX and α -glucosidase inhibitory activities under the applied experimental conditions. These results indicate that the biological activities of pyrolysis-derived fractions are not determined solely by TPC, TFC, or TAC values, but also by the structural diversity and reactivity of the compounds enriched in each fraction. Therefore, PHF appears to be particularly promising for further investigation as a pyrolysis-derived bioactive fraction with anti-inflammatory and antidiabetic potential.

Evaluation of the antibacterial activities

The increasing prevalence of antibiotic-resistant bacterial strains has intensified the search for natural antibacterial agents with alternative modes of action (Lai et al. 2021; Dönmez and Önem 2024). In this context, bark-derived phenolics, resins, and other secondary metabolites from *Pinus* species are of interest because they are associated with plant defense responses and may contribute to antibacterial activity

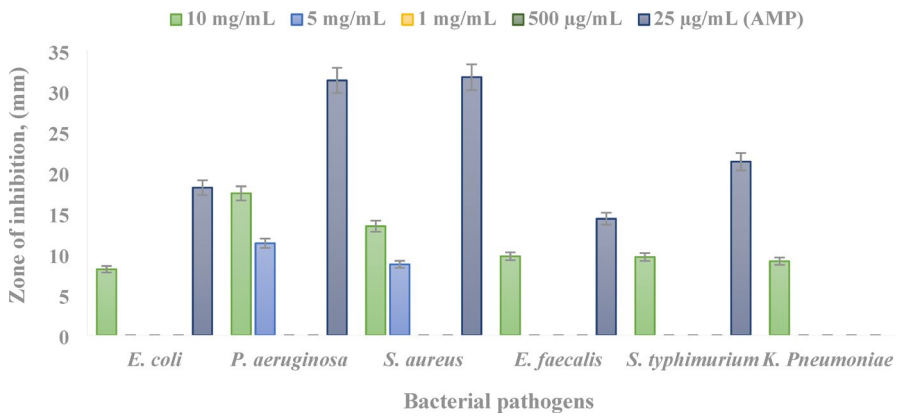


Fig. 2 Mean inhibition zone diameters (zone of inhibition, mm) of the PHF against various pathogens at different concentrations. Ampicillin (AMP, 25 µg/mL) was used as the positive control. All data are expressed as mean±SD ($n=3$), with statistically significant differences at $p<0.05$

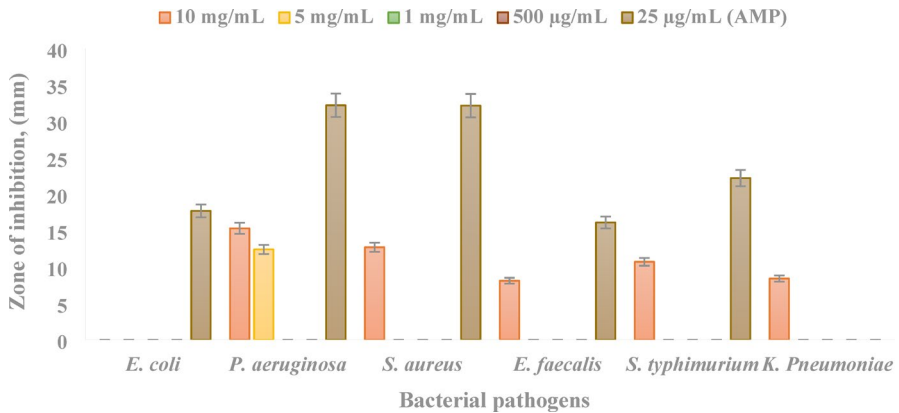


Fig. 3 Mean inhibition zone diameters (zone of inhibition, mm) of the PLF against various pathogens at different concentrations. Ampicillin (AMP, 25 µg/mL) was used as the positive control. All data are expressed as mean±SD ($n=3$), with statistically significant differences at $p<0.05$

(Keita et al. 2022; Truzzi et al. 2025). In the present study, the antibacterial activities of PLF and PHF were evaluated by the agar well diffusion method against *E. coli*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *S. typhimurium*, and *K. pneumoniae*. These strains were selected to represent clinically relevant Gram-negative and Gram-positive bacteria with different cell wall structures and resistance-related characteristics (Tong et al. 2015; WHO, 2017; Wyres and Holt 2018).

The antibacterial activities were examined at concentrations ranging from 0.5 to 10 mg/mL, and inhibition zones were measured in millimeters. The results are presented in Fig. 2 for PHF and Fig. 3 for PLF, while representative Petri dish images are provided in Figures S1 and S2, respectively. Both pyrolysis-derived fractions showed concentration-dependent antibacterial activity, with more pronounced inhibition generally observed at 5 and 10 mg/mL. The activity varied depending on both the bacte-

rial strain and the fraction type. PHF produced measurable inhibition zones against several tested strains, particularly at 10 mg/mL. The strongest activity was observed against *P. aeruginosa* (17.40 ± 1.20 mm), followed by *S. aureus* (13.40 ± 0.90 mm) (Fig. 2). The inhibition of *P. aeruginosa* is noteworthy because this Gram-negative bacterium is known for its intrinsic resistance, which is partly associated with its outer membrane barrier and efflux pump systems (Pang et al. 2019). The antibacterial activity of PHF may be related to phenolic-rich, resinous, and lipophilic constituents generated during pyrolysis, which can interact with bacterial membranes and increase membrane permeability. Phenolic compounds have been reported to affect bacterial viability by increasing membrane permeability, disrupting protein function, and promoting oxidative stress-related damage (Zhao et al. 2025).

PLF also showed antibacterial activity, particularly at 10 mg/mL, with inhibition zones against *P. aeruginosa* (15.30 ± 1.00 mm), *S. aureus* (12.70 ± 0.90 mm), and *S. typhimurium* (10.70 ± 0.80 mm) (Fig. 3). Since PLF is expected to contain more volatile and lower molecular weight constituents, including terpene-like compounds, its antibacterial effect may be associated with membrane-related interactions. Terpenes and related volatile constituents have been reported to exert antibacterial effects by disrupting membrane integrity, especially in Gram-positive bacteria (Hajdari et al. 2016; Virjamo and Julkunen-Tiitto 2018). However, the generally stronger activity of PHF suggests that the heavier fraction may contain chemically more complex and biologically more active antibacterial constituents.

An important observation in this study was that PLF and PHF produced inhibition zones against certain strains under conditions where ampicillin showed no detectable activity. In particular, both pyrolysis-derived fractions inhibited *K. pneumoniae*, whereas ampicillin was ineffective under the applied assay conditions. Since β -lactam resistance may involve mechanisms such as β -lactamase production, porin changes, efflux activity, and altered penicillin-binding proteins, this result suggests that the multicomponent structure of PLF and PHF may provide antibacterial effects through mechanisms different from those of β -lactam antibiotics (Eiamphungporn et al. 2018; Mondemé et al. 2023; Elshobary et al. 2025). However, further mechanistic studies are required to identify the specific compounds and cellular targets responsible for this activity. The broader activity observed for these fractions is consistent with reports indicating that complex plant-derived mixtures may display flexible antibacterial responses compared with single-target antibiotics (Woo et al. 2023; Zouine et al. 2024).

To the best of our knowledge, the antibacterial activities of PLF and PHF obtained from *P. sylvestris* bark by pyrolysis have not been previously reported against the tested bacterial strains. Most studies on *Pinus*-derived antibacterial products have focused on solvent-based extracts from bark or woody tissues (Sharma et al. 2015; Ramos et al. 2022; Bhardwaj et al. 2022). Dönmez and Önem (2024) reported that acetone: water extracts of *P. brutia* and *P. nigra* bark showed activity against *S. aureus* at 30 mg/mL, with inhibition zones of 20.0 and 15.7 mm, respectively, whereas no activity was observed against *E. coli*. In contrast, in the present study, PLF and PHF exhibited measurable activity against both Gram-positive bacteria (*S. aureus* and *E. faecalis*) and Gram-negative bacteria, including *E. coli*, *K. pneumoniae*, and *S. typhimurium* (Figs. 2 and 3). This indicates that pyrolysis-derived

fractions may provide a broader antibacterial profile than some conventional solvent extracts under the tested conditions. Similarly, Dziedzinski et al. (2020) reported that aqueous extracts of *P. sylvestris* shoots showed strong antibacterial activity against *P. aeruginosa* (27 mm) and *K. pneumoniae* (22 mm) at approximately 33 mg/mL. In the present study, PLF and PHF produced inhibition zones against Gram-negative bacteria at lower tested concentrations, particularly 5 and 10 mg/mL. Although direct comparison is limited by differences in plant material, extraction method, concentration, and assay conditions, these findings support the antibacterial potential of *P. sylvestris*-derived products. Overall, the results indicate that pyrolysis-derived PLF and especially PHF from *P. sylvestris* bark possess notable antibacterial activity against clinically relevant bacterial strains. These findings support the potential of pyrolysis-based biomass valorization not only for chemical recovery but also for producing biologically active, high-value fractions from forestry waste.

Phenolic and flavonoid profiling of the PHF using LC-MS/MS

Based on the comparative biological activity results, PHF was selected for detailed phytochemical characterization because it showed stronger anti-inflammatory and antidiabetic activities and generally more pronounced antibacterial responses than PLF. Therefore, the phenolic profile of PHF was investigated using LC-MS/MS. This technique provides sensitive and selective identification and quantification of phenolic compounds in plant-derived and lignocellulosic matrices (López-Fernández et al. 2020). Quantification was performed using calibration curves prepared from analytical standards in the range of 0.25–8 ppm. The linear regression coefficients ranged from $R^2 = 0.9934$ to 0.9996, and the %RSD values were below 10%, indicating acceptable linearity, sensitivity, and repeatability according to commonly used analytical criteria (Myrtsi et al. 2021).

Among the 31 reference standards screened, only four phytochemical compounds were detected and quantified in PHF within the targeted LC-MS/MS screening panel used in this study. The results for all target analytes are presented in Table 5, while the molecular structures of the identified compounds are shown in Fig. 4. Caffeic acid (7190.65 $\mu\text{g/g}$) and protocatechuic acid (2694.30 $\mu\text{g/g}$) were the dominant phenolic compounds in PHF. Protocatechuic aldehyde was also detected at 777.24 $\mu\text{g/g}$, whereas rutin was present at a trace level (0.42 $\mu\text{g/g}$). The remaining target compounds were below the limit of detection (LOD) or limit of quantification (LOQ), indicating that they were not detectable or not quantifiable with sufficient analytical reliability in this sample. The low abundance or absence of several flavonoids, such as quercetin, luteolin, and naringenin, may be related to their thermal sensitivity during pyrolysis. In contrast, phenolic acids and related aromatic structures appear to be more stable under the applied pyrolysis conditions, which is consistent with the high TPC value observed for PHF.

The predominance of caffeic acid and protocatechuic acid is particularly relevant because both compounds have been associated with various biological activities. Protocatechuic acid, a hydroxybenzoic acid derivative, has been reported to exhibit anti-inflammatory, antibacterial, hepatoprotective, antiviral, and other bioactive properties in *in vitro* and *in vivo* studies (Taofiq et al. 2019; Ferreira-Santos et al.

2020a). Caffeic acid, a hydroxycinnamic acid derivative, is also known for its antioxidant and photoprotective properties (Ferreira-Santos et al. 2020a, b). Commercial pine bark extracts such as Pycnogenol® and Oligopin® have also been reported to contain phenolic acids, including protocatechuic and caffeic acids, and are associated with antioxidant and anti-inflammatory effects (Ustun et al. 2012; Segal et al. 2018). In addition, protocatechuic aldehyde, which may occur naturally or arise from the degradation of phenolic acids, has been linked to antiadipogenic, antiproliferative, and anti-inflammatory effects (Zhang et al. 2015; Chang et al. 2021). Although rutin was detected only at a low concentration, rutin-containing polyphenolic systems have been reported to contribute to antioxidant, anti-inflammatory, and antimicrobial activities (Cabral et al. 2020; Jesus et al. 2020).

To the best of our knowledge, this is the first report describing the LC–MS/MS-based phenolic profile of PHF obtained from *P. sylvestris* bark through pyrolysis. Previous studies have mainly focused on solvent-extracted phenolic profiles of different *Pinus* species and plant parts. Dziedzinski et al. (2020) reported gallic, caffeic, syringic, *p*-coumaric, chlorogenic, sinapic, trans-cinnamic, vanillic, and salicylic acids, together with several flavonoids, in *P. sylvestris* cone extracts analyzed by UPLC–Q-TOF-MS. Ferreira-Santos et al. (2020a) also reported *p*-coumaric, ferulic, and caffeic acids as common hydroxycinnamic acids in pine-derived extracts. Similarly, Kivrak et al. (2013) identified 15 phenolic compounds, including gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and several flavonoids, in *P. brutia* bark extracts using UPLC–ESI–MS/MS. The repeated detection of protocatechuic acid, caffeic acid, and rutin in different *Pinus* matrices suggests that these compounds are characteristic phenolics of the genus, although their levels may vary depending on species, geographical origin, plant part, extraction method, and solvent system (Ku and Mun 2008; Lantto et al. 2009; Ferreira-Santos et al. 2019, 2020a).

Overall, LC–MS/MS analysis showed that PHF is mainly characterized by phenolic acids, particularly caffeic acid and protocatechuic acid, together with protocatechuic aldehyde and trace rutin. These results support the biological activity profile observed for PHF and indicate that pyrolysis-based fractionation can yield phenolic-rich products from *P. sylvestris* bark waste. However, further studies are required to determine whether the detected compounds act individually or synergistically in the observed bioactivities.

Conclusion

This study demonstrates that pyrolysis-based fractionation of *P. sylvestris* bark yields two chemically and biologically distinct fractions with different functional potentials. Overall, PLF was characterized by a stronger antioxidant profile, whereas PHF showed more pronounced anti-inflammatory, antidiabetic, and antibacterial activities. These findings indicate that the biological performance of pyrolysis-derived fractions is influenced not only by the total levels of phenolics or flavonoids, but also by differences in their structural and compositional features. The results further suggest that pyrolysis may serve as an effective and sustainable valorization strategy for converting *P. sylvestris* bark, an underutilized forest by-product, into value-added

Table 5 Quantitative determination of phenolic compounds in PHF by LC–MS/MS

No	Compounds	Chemical Formula	RT ^a	µg compounds/ g extract	%RSD ^b	Parent ion (m/z)	Main fragment ions (m/z)	Ion mode	LOD/LOQ (µg/g)
1	Pyrogallol	C ₆ H ₆ O ₃	7.30	ND	–	124.86	69.31 79.27	Negative	0.10/0.30
2	Gallic acid	C ₇ H ₆ O ₅	9.24	ND	–	169.70	80.50 126.20	Negative	0.08/0.25
3	Protocatechuic acid	C ₇ H ₆ O ₄	11.16	2694.30	3.2	155.01	65.40 93.20	Positive	0.08/0.25
4	Protocatechuic aldehyde	C ₇ H ₆ O ₃	12.03	777.24	4.5	136.90	92.25 108.20	Negative	0.10/0.32
5	Sesamol	C ₇ H ₆ O ₃	12.65	ND	–	137.18	108.17 109.29	Negative	0.12/0.38
6	Gentisic acid	C ₇ H ₆ O ₄	12.48	ND	–	153.70	109.50	Negative	0.09/0.28
7	Catechin	C ₁₅ H ₁₄ O ₆	12.54	ND	–	289.20	203.90 245.70	Negative	0.20/0.60
8	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	13.69	ND	–	353.40	86.50 192.10	Negative	0.15/0.45
9	Epicatechin	C ₁₅ H ₁₄ O ₆	13.95	ND	–	291.50	123.30 139.30	Positive	0.18/0.55
10	Caffeic acid	C ₉ H ₈ O ₄	14.32	7190.65	2.7	179.70	135.20 136.20	Negative	0.05/0.18
11	Vanillin	C ₈ H ₈ O ₃	14.67	ND	–	150.91	92.30 136.10	Negative	0.10/0.30
12	Syringic acid	C ₉ H ₁₀ O ₅	17.04	ND	–	183.07	77.30 123.20	Negative	0.12/0.35
13	Syringic aldehyde	C ₉ H ₁₀ O ₄	15.20	ND	–	180.88	151.09 166.09	Negative	0.14/0.42
14	Taxifolin	C ₁₅ H ₁₂ O ₇	15.75	ND	–	303.0	126.20 285.50	Negative	0.20/0.65
15	<i>p</i> -Coumaric Acid	C ₉ H ₈ O ₃	15.89	ND	–	163.90	94.30 120.20	Negative	0.10/0.30

Table 5 (continued)

No	Compounds	Chemical Formula	RT ^a	µg compounds / g extract	%RSD ^b	Parent ion (m/z)	Main fragment ions (m/z)	Ion mode	LOD/LOQ (µg/g)
16	Vitexin	C ₂₁ H ₂₀ O ₁₀	16.28	ND	–	430.90	283.03 311.10	Negative	0.25/0.75
17	Ferulic acid	C ₁₀ H ₁₀ O ₄	16.16	ND	–	195.02	89.40 177.40	Positive	0.11/0.33
18	4-Hydroxybenzoic acid	C ₇ H ₆ O ₃	16.58	ND	–	137.90	66.60 94.60	Negative	0.09/0.27
19	Salicylic acid	C ₇ H ₆ O ₃	16.59	ND	–	137.14	65.50 93.26	Negative	0.08/0.25
20	Hesperidin	C ₂₈ H ₃₄ O ₁₅	16.81	ND	–	608.78	301.34 164.01	Negative	0.30/0.90
21	Rosmarinic acid	C ₁₈ H ₁₆ O ₈	17.07	ND	–	359.18	134.30 162.20	Negative	0.18/0.55
22	Oleuropein	C ₂₅ H ₃₂ O ₁₃	16.98	ND	–	539.10	275.80 377.50	Negative	0.35/1.05
23	Luteolin-7-glucoside	C ₂₁ H ₂₀ O ₁₁	17.33	ND	–	446.89	284.00 285.00	Negative	0.28/0.85
24	Rutin	C ₂₇ H ₃₀ O ₁₆	17.60	0.42	8.9	609.37	300.60 301.70	Negative	0.15/0.45
25	Resveratrol	C ₁₄ H ₁₂ O ₃	17.62	ND	–	228.98	107.20 135.10	Positive	0.12/0.36
26	Naringenin	C ₁₅ H ₁₂ O ₅	19.20	ND	–	273.00	147.10 153.00	Positive	0.20/0.60
27	Quercetin	C ₁₅ H ₁₀ O ₇	19.86	ND	–	301.00	152.10 179.90	Negative	0.22/0.66
28	Apigenin	C ₁₅ H ₁₀ O ₅	21.25	ND	–	268.86	117.19 149.11	Negative	0.24/0.72
29	Chrysin	C ₁₅ H ₁₀ O ₄	22.70	ND	–	254.97	153.10 103.28	Positive	0.26/0.78

Table 5 (continued)

No	Compounds	Chemical Formula	RT ^a	µg compounds/ g extract	%RSD ^b	Parent ion (m/z)	Main fragment ions (m/z)	Ion mode	LOD/LOQ (µg/g)
30	Galangin	C ₁₅ H ₁₀ O ₅	22.83	ND	–	268.85	169.15 171.08	Negative	0.25/0.75
31	Flavone	C ₁₅ H ₁₀ O ₂	22.96	ND	–	222.90	77.28 121.15	Positive	0.23/0.69

^a RT: Retention time (min);^b %RSD: relative standard deviation;

ND Below the LOD under the applied LC-MS/MS conditions, LOD limit of detection, LOQ limit of quantification

%RSD values were calculated only for compounds quantified above the LOQ

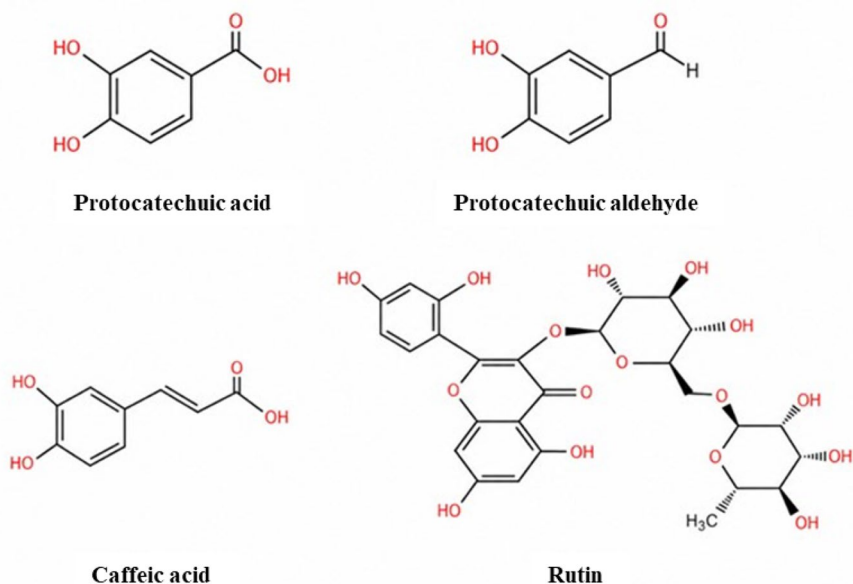


Fig. 4 Chemical structures of phenolic compounds identified in the PHF fraction by LC-MS/MS analysis

bioactive fractions. In particular, the stronger bioactivity observed for PHF highlights the potential importance of more complex phenolic and pyrolysis-derived constituents in shaping enzyme inhibitory and antibacterial effects. Thus, beyond simple extraction, pyrolysis appears to offer a functional transformation route capable of generating fractions with differentiated biological applications. Taken together, the present findings support the potential of pyrolysis-derived bark fractions as valuable agents for applications focused on antioxidant, anti-inflammatory, antidiabetic, and antimicrobial activities. Nevertheless, further studies aimed at identifying the specific bioactive compounds responsible for the observed effects, clarifying their molecular mechanisms, and validating these results in more advanced experimental models are essential to establish a comprehensive foundation for their therapeutic use.

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Data availability Upon reasonable request, the datasets that were obtained and/or analyzed throughout the current investigation are eligible to be provided by the corresponding author.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This article does not contain any studies with animals or human participants.

Consent to Participate The authors agreed to participate in this work.

Consent for publication The authors agreed to publish this work.

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