Bark of Protium tenuifolium Engl from the Amazonian biome as a source of phenolic extracts with antioxidant properties

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Abstract

This study evaluated the anatomical and chemical components of the bark of *Protium tenuifolium*, a tree species from the Brazilian Amazon, to provide information supporting its value in various industries as a source of polyphenols and natural antioxidants. The bark anatomy was analysed, as was the overall chemical composition (extractives, lignin, suberin, ash and polysaccharides), and a quantitative elemental analysis was performed of the bark and two tannin extracts (extracted with water only and with a mixture of water and Na_2SO_3). The phenolic composition and antioxidant activity of the bark extracts were quantified. The results indicated that the bark is composed of conductive phloem, nonconductive phloem and rhytidome. The average chemical composition was 15.9% extractives, 35.3% total lignin, 1.9% suberin, 15.4% ash and 31.5% polysaccharides. The condensed tannin yield in the extract obtained with water alone was 5.1%, and that in the treatment with water and Na_2SO_3 was 8.1%. The ethanol-water extract had a high phenolic content (112.6 mg GAE g^{-1} extract). The bark extract showed strong antioxidant activity, reaching 83.5% inhibition of DPPH free radicals, which was higher than the value of 75.0% for the commercial antioxidant BHT at the same concentration. These results demonstrate that *Protium tenuifolium* is a potential natural Amazonian source of phenolic compounds and antioxidants and can be used for medicinal purposes and the production of various sustainable products, such as cosmetics. The extraction of these compounds adds substantial value to the bark of this Amazonian species.

Introduction

Currently, bark is used to produce products with high added value, such as biocomposites prepared from bark extracts; in particular, polyphenols have ample potential for different applications (Souza et al. 2021).

The biological effects of polyphenols mainly include the neutralization of highly reactive species, such as superoxide (O_2^-) and hydroxyl (OH^-) radicals produced due to oxidative stress, the activation of antioxidant enzymes and improvements in the oxidative stress response (Carocho et al. 2018). The most common plant antioxidants are tannins, flavonoids, cinnamic acid derivatives, coumarins and organic acids (Farias et al. 2013).

The increased demand for plant-based antioxidants has intensified in recent decades because research has shown that long-term intake of synthetic antioxidants causes health problems such as gastrointestinal issues, skin allergies and increased risk of cancer (Engin et al. 2011; Lourenço et. 2019). High doses of synthetic antioxidants can cause DNA damage (Kornienko et al. 2019). The synthetic antioxidant butylated hydroxytoluene (BHT) has already been considered responsible for adverse effects on the liver and cancer in animals (Botterweck et al. 2000; Saad et al. 2007).

The intensified search for phenolic compounds can also be attributed to their potential for numerous commercial applications, such as food dyes, bioactive packaging, cosmetic products, textiles, rubber,

plastics, foams, medicines (Farrag et al. 2018; Mendoza et al. 2018; Chen et al. 2020; Araujo et al. 2021) and water treatment (SÁnchez-Martín et al. 2010; Bello et al. 2020). Although there are several advantages to using bark, its industrial use is still limited, mainly due to the lack of knowledge about the specific characteristics of forest species (Jansone et al. 2017).

Most studies on bark composition have considered species from temperate climates (Souza et al. 2021b). In Brazil, the main research considers species from the Cerrado and Caatinga biomes, and limited information is available on the bark of the Amazon biome although scientific information is critical for the valuation of species in the region and the future production of sustainable products with polyphenols from Amazonian bark (Araujo et al. 2020).

The family Burseraceae comprises approximately 700 species; the genus *Protium* Burm. f. alone includes 68 species found in Brazil (Santana et al. 2020). Approximately 40 species are endemic to the Amazon region (Daly, 1992). The species *Protium tenuifolium*, popularly known as breu, is distributed throughout the Amazon biome (Flora do Brasil, 2022).

P. altsonii is used to produce wood for industrial use; however, it is not a widely exploited species in the Amazon (Batista, 2018). It was reported that the chemical composition of resins from this species typically includes limonene, p-cymene, and trans-dihydro-α-terpineol (Murthy et al., 2016). Silva (2012) analysed the essential oil of the wood of this species and observed that the predominant constituents were sesquiterpenes and the major components were α-calacorene and epi-α-muurolol.

Species of the genus *Protium* have great potential for the production of new products because they produce oleoresins, popularly known as breu. They are used in traditional medicine by Amazonian populations, as is the case for the quilombola communities that live around the Erepecuru River in Oriximiná, Pará. Oleoresins are used as anti-inflammatories, analgesics, expectorants, and natural insecticides, as well as for incense and for caulking boats. Oleoresins are used in industry mainly for the preparation of perfumes (Silva et al. 2017; Ferreira et al. 2020).

Santos et al. (2021) studied the essential oil of the bark of *P. tenuifolium* and obtained a yield of 0.05%. GC/MS analysis indicated that the main chemical constituents of the oil were limonene (56.17%), α-phellandrene (16.22%) and ρ-cymene (10.52%).

The bark of *P. tenuifolium* was analysed in this study for the first time to investigate its anatomical characteristics, overall chemistry and tannin extracts and the antioxidant properties of its polar extractives, with the objective of obtaining information to add value and expand the direct uses for the bark of this species.

Materials And Methods

(1)

$$\mathrm{TS} = \left(\frac{drymassoftheextract}{wetmassoftheextract} \right) * 100$$

(2)

$$\mathrm{TSY} = \left(\frac{TS\% * totalmassoftheconcentrate}{100} \right) * 100$$

The Stiasny number (SN) was determined in duplicate. For this purpose, 20 g of concentrated extract, 10 mL of deionized water, 2 mL of 10 mol L^{-1} HCl and 4 mL of 37% formaldehyde were successively placed in a volumetric flask and heated in a reflux system for 35 minutes. Timing was initiated after boiling. The precipitate was filtered through a sintered glass crucible with porosity 2 and dried in a hot air oven at 103 ± 3°C until constant mass. The Stiasny number was calculated according to Eq. 3. The gravimetric yield of condensed tannins (GYCT) was determined by Eq. 4. The yield of nontannic components (YNTC) was obtained by the difference between YNTC and GYCT.

(3)

$$\mathrm{Stiasnynumber} = \left(\frac{drymassoftheprecipitate}{drymassof20gofextract} \right) * 100$$

(4)

$$GYCT = \frac{SN\% * YNTC}{100}$$

Phenolic compounds from tree bark extract

Bark samples of 0.5 g (dry basis) were extracted in an ethanol-water solution with a 1:1 ratio (v/v) using an ultrasonic bath at 50°C for 1 h. The solution was filtered through a sintered glass crucible with porosity 2 under vacuum conditions, and the volume of the filtrate was brought to 50 mL with deionized water. This extract was used to determine the contents of total phenols, total flavonoids and condensed tannins. The solid residue retained in the filter was dried, and the extraction yield relative to the initial mass of the material was calculated. The total phenol content was determined by the Folin-Ciocalteu method using gallic acid as the standard (Singleton et al. 1965). The total flavonoids were quantified by an aluminium chloride colorimetric assay with catechin as the standard (Zhishen et al. 1999). The content of condensed tannins was determined by the vanillin H_2SO_4 method (Abdalla et al. 2014). The absorbance readings were performed using an SP-22 Biospectro SP-22 spectrophotometer, and the results are expressed in milligrams of gallic acid equivalents (GAE; total phenols) or catechin equivalents (CE; flavonoids and condensed tannins)/g of dry extract.

High-performance liquid chromatography (HPLC) was used to quantify the phenolic compounds in the samples and establish a profile of the compounds present. The standards used to analyse phenolic compounds were trigonelline, gallic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, vanillin, p-coumaric acid, ferulic acid, m-coumaric acid, o-coumaric acid, resveratrol, quercertin, rosmarinic acid, transcinamic acid and rutin. For the chromatographic analyses, samples and solvents were filtered using 0.45 µm membranes, as detailed in arauj et al. (2020).

The samples were quantified by the external standardization method, and the analytical curves were constructed by diluting stock solutions containing 1000 mg L^{-1} of each standard dissolved in methanol. The analytical curves were obtained by linear regression, and the coefficient of determination R^2 = 0.99.

The analyses were performed in a Shimadzu® high-performance chromatograph equipped with four LC-20AT high-pressure pumps, an SPD-M20A diode array detector, a DGU-20A5 degasser, a CBM-20A interface, a CTO-20AC oven and an automatic injector with an SIL-20A autosampler. Separation was performed using a Shimadzu®-Shim-pack GVP-ODS-C18 column (4.6 x 250 mm, 5 µm) connected to a Shimadzu®-Shim-pack GVP-ODS-C18 precolumn (4.6 x 10 mm, 5 µm). The elution solvents used were as follows: Phase A, 2% acetic acid solution in ultrapure water obtained from a Milli-Q Integral Water Purification System; Phase B, methanol:water:acetic acid (70:20:2% v/v). The wavelength was 280 nm, the flow was 1.0 mL min^{-1}, the column temperature was 35°C and the injection volume was 20 µL. To calculate the concentration of phenolic compounds, the equations of the straight line specific for each standard were used.

Antioxidant activity of bark extract

The analysis was performed according to the method described by Teixeira (2012) with the modifications of Araujo et al. (2020). The extracts from the analysis of phenolic compounds were lyophilized and used to determine the antioxidant activity by stabilizing the free radical of 2,2-diphenyl-1 picrylhydrazyl hydrate (DPPH). Samples of 0.0125 g (lyophilized bark and BHT extracts) were solubilized in 25 mL of CH_3CH_2OH, subsequently diluted to concentrations of 25, 50, 100, 150, 200, 250 and 500 µg mL^{-1} in CH_3CH_2OH and kept under refrigeration overnight.

An ethanolic solution of DPPH with a concentration of 40 mg L^{-1} was prepared and kept away from light. Samples (0.3 mL) were added to the test tubes at concentrations of 25, 50, 100, 150, 200, 250 and 500 µg mL^{-1}, followed by the addition of 2.7 mL of DPPH solution. Negative and blank controls were prepared at the same time. The negative control was prepared by adding 0.3 mL of C_2H_5OH and 2.7 mL of DPPH solution, and the blank (for each concentration) was prepared by adding 2.7 mL of ethanol and 0.3 mL of sample solution. The solutions remained in the dark for 60 minutes. After this, the absorbance readings were performed in a UV/Vis spectrophotometer (Shimadzu UV-1601 PC) at 515 nm. The standard comparison used in the analysis was the synthetic antioxidant BHT, and all analyses were performed in triplicate. The antioxidant activity was expressed as the percentage of inhibition, which was calculated

from the absorbance values according to Eq. 5 by plotting the inhibition data relative to the sample concentrations. The IC_{50} value expresses the antioxidant concentration necessary to reduce the presence of the free radical DPPH by 50%.

(5)

$$Inhibition\,(\%) = \frac{^{A}DPPH - ^{A}Sample}{^{A}DPPH} x100$$

Statistical analysis

Statistical analysis was conducted in a completely randomized design using *OriginPro software*, version 2018 SR1, OriginLab Corporation, Northampton, MA, USA. The Tukey test was applied to verify the differences between extraction conditions in both cases (with and without Na_2SO_3). A significance level of 5% ($p < 0.05$) was adopted for all statistical analyses.

Results And Discussion

Anatomical structure of the bark

The average thickness of the *P. tenuifolium* bark is 7.4 mm. The surface is scaly and has a black colour, and its interior is carmine red (Fig. 2A). The bark includes the phloem (conductive and nonconductive) and rhytidome (Fig. 2B).

The periderm is developed in continuous sequential layers with some sclerified phellem and phelloderm cells (Fig. 3A). The transition from conductive to nonconductive phloem occurs gradually (Fig. 3C). The conductive phloem is wide and includes solitary tube elements in small groups with small companion cells adjacent to the fibres and axial and radial parenchyma cells (Fig. 3B, D). The tube elements have inclined and scalariform plates, tangential diameters of 6-19 µm, and lengths of 185-626 µm, on average (Fig. 4.C). The tube elements are accompanied by a companion cell and are arranged in radial rows in the conducting phloem (3.BD).

The number of ray cells varied from 9-27 vertically and from 2-6 horizontally. The mean ray height was 95-315 µm, and the width was 11-71 µm, consisting of procumbent cells (Fig. 4.A).

The fibres have an average length of 593-1,049 µm and an average thickness of 8.8 µm. In the conductive phloem, the fibres occur in small isolated groups between the parenchyma cells (Fig. 3D). The sclereids have lignified walls and reach an average tangential diameter of 11-30 µm (Fig. 3A). A large number of secretory cells are present in the phloem (Fig. 3A, B and D). According to Metcalfe and Chalk (1950), the presence of secretory channels of aromatic resin associated with the phloem is characteristic of many Burseraceae species. *Protium heptaphyllum* and *P. tenuifolium* both exhibit secretory cells in the phloem (Palermo et al., 2018).

Overall chemical composition of the bark

Table 1 summarizes the overall chemical composition of *P. tenuifolium* bark. The content of total extractives was 15.9%, of which approximately 94.3% corresponded to polar extractives.

Table 1 Overall chemical composition (% of total dry mass) of *Protium tenuifolium* bark

Chemical components	% Standard deviation
Extractives	
Dichloromethane	0.9 (0.3)
Ethanol	8.4 (0.1)
Water	6.6 (0.3)
Total	15.9
Lignin	
Klason lignin	32.9 (0.3)
Soluble lignin	2.4 (0.1)
Total	35.3
Suberin	1.9 (1.1)
Ash	15.4 (0.10)
Polysaccharides	31.5

The values in parentheses correspond to the standard deviation.

The chemical composition of bark is complex and varies between the internal and external bark, as well as between and within species. The bark differs from the wood in terms of chemical components due to the high content of water-soluble extractives or the use of organic solvents, as seen in Table 1 (Fradinho et al. 2002).

The total extractives content is similar to that of the bark of *Eucalyptus urophylla* clones (14.3% to 17.6%) (Sartori et al. 2016) and *Tachigali glauca* (15.3%) (Mota et al. 2021b), greater than that of the bark of *A. lecointei* from the Amazon (12.9%) (Mota et al. 2021a) and lower than that of *Tachigali guianensis* (18.0%) (Mota et al. 2021b). Given that the total content of bark extractives is 20-40%, these extractives can be considered a potential raw material for refining; bark contains two to six times more extractives than stem wood and is an abundant raw material but is not valued by industry for biorefining (Routa et al. 2020).

Deineko and Faustova (2015) investigated the outer bark of poplar and observed a lignin concentration of more than 20%.The bark of *Protium tenuifolium* is quite lignified (35.3%) compared with the bark of other Amazonian species, such as *Copaifera langsdorffii* (36.6% lignin content) (Carmo et al. 2016a) and *Albizia niopoides* (37.1%) (Carmo et al. 2016b). Some Brazilian species showed lower values, such as *Myrcia eximia* with 21.1% (Araujo et al. 2020) and *Myracrodruon urundeuva* with 25.74% (Sousa et al. 2020).

The high observed lignin content is in agreement with the large proportion of highly lignified sclereids (Fig. 3A). This high lignin content can be used in industry for the production of biofuels (Moniz et al. 2015). Notably, several studies have shown that modified lignin has multiple applications, and several studies have also shown the potential of depolymerized lignin for the production of valuable chemicals (Chio et al. 2019).

Polysaccharides represented 31.5% of the *P. tenuifolium* bark. This value is lower than the contents of 39.67% reported for *Betula celtiberica* (Sillero et al. 2019) and 41.13% for *Quercus faginea* (Ferreira et al. 2018). The observed value is also higher than the content of 25.9% found in the Amazonian species *Goupia glabra* (Carmo et al. 2016c). Polysaccharides have been researched globally due to their broad biological activities, such as anti-inflammatory, immunoregulatory, antitumour, anticoagulation and antioxidant activities (Song et al.2021).

The suberin content was low (1.9%). Phellem cells are dead cells characterized by a cell wall containing suberin that is internally deposited in the primary cell wall (Leite and Pereira 2017). When the proportion of phellem is small, the suberin content is low. In *T. grandis*, 1.9% suberin was observed, a value similar to that found in *P. tenuifolium* (Baptista *et al.* 2013). In phloem tissues, suberin is often accompanied by suberin-associated waxes, such as in the cuticle; these waxes are nonpolymerized compounds of variable composition, including fatty alcohols, fatty acids, alkanes, monoacylglycerols and other compounds, such as sterols; in the bark of *P. tenuifolium,* the amount of suberin quantified was low (Serra and Geldner, 2022).

The ash content was 15.4, which is high compared to the bark ash contents of other species, such as *Eucalyptus globulus* (3.5%) (Gominho et al. 2021), *A. peregrina* (8.2%) and *A. colubrina* (7.7%) (Mota et al. 2017). In *Tectona grandis*, a value of 18.5% was observed, higher than that found in *P. tenuifolium* (Baptista *et al.* 2013).

Quantitative elemental analysis of the bark

The elemental composition of the mineral components of the bark of *P. tenuifolium* is shown in Table 2. Iron had the highest concentration (74.5%), followed by calcium (64.2%), nitrogen (17.1%), manganese (13.2%) and potassium (11.9%).

Table 2 Elemental mineral components of *Protium tenuifolium* bark.

Minerals	mg/100 g bark	% of bark
N	6.9	17.1
P	0.2	0.5
K	4.8	11.9
Ca	26.3	64.2
Mg	0.9	2.2
S	1.4	3.5
Minerals	µg/100 g bark	% of bark
Mn	24.4	13.2
Zn	5.9	3.2
B	9.9	5.4
Cu	6.8	3.7
Fe	137.3	74.5

The high amount of calcium may have caused the frequent crystals of calcium oxalate observed in parenchyma cells (Fig. 4B), which are also observed in other Amazonian species, such as *Astronium lecointei* (Mota et al. 2021a) and *Goupia glabra* (Carmo et al. 2016c). Calcium and potassium are also found in the bark of the Brazilian species *Myracrodruon urundeuva* (Sousa et al. 2020). The large amounts of Fe, Ca, N, Mn and K indicate that *P. tenuifolium* can be used as a plant substrate.

Condensed bark tannins

The mean values for tannins extracted in water and in Na_2SO_3 solution from the bark of *P. tenuifolium* are shown in Table 3. The addition of sodium sulfite corresponded to an increase in total solids yield of 3.8% and an increase of 3.0% in condensed tannins.

Table 3 Mean values of total solids yield (TSY), Stiasny number (SN), condensed tannin yield (CTY), and yield of nontannic compounds (YNTC) of tannins extracted in water with and without the addition of 3% Na_2SO_3.

Species	Treatment	TSY (%)	SN (%)	CTY (%)	YNTC (%)
P. tenuifolium	Water	7.8 (0.1)B	64.8 (2.1)B	5.1 (27.7)B	2.74 (0.1)B
	Water and Na_2SO_3	11.6 (1.3)A	71.2 (22.2)B	8.1 (11.5)A	4.26 (2.7)A

Values followed by the same letter in the column do not differ statistically based on the Tukey test at the 5% significance level. The values in parentheses correspond to the standard deviation of 6 samples.

The total yield of solids, condensed tannins yield and yield of nontannic compounds extracted in water with and without the addition of 3% Na_2SO_3 differed significantly according to Table 3: the added Na_2SO_3 significantly increased these values in the latter treatment. An increase in these parameters was also observed in the Amazon species *Myrcia eximia* with extraction in water and 3% Na_2SO_3 (Araujo et al. 2021).

Several studies have reported increased yields of condensed tannins when extracted in sodium sulfite solution. Hoong et al. (2009) used Na_2SO_3 in the extraction of tannins from *Acacia mangium* and obtained a yield between 15% and 25%; Mori et al. (2003) used a 3% solution of Na_2SO_3 and recorded an increase in condensed tannins of 34.9% in the bark of barbatimão (*Stryphnodendron adstringens*); *P. tenuifolium* extraction with 3% Na_2SO_3 solution showed a yield of 8.1% (Table 3). This increase in yield occurs because the salt (sodium sulfite) makes tannins more soluble during water extraction by opening the heterocyclic ring of the flavonoid of the condensed tannin unit (Pizzi and Mittal, 1994).

Lins et al. (2019) obtained a tannin yield of 4.02% for water extracts of *Erythrina velutina*, a value close to that observed in *P. tenuifolium.* Determination of the Stiasny number is one of the most commonly used methods for the quantification of tannins. The relevant method is based on a chemical reaction that occurs during the precipitation of flavanol tannins through condensation with formaldehyde in acid. These products have a high molecular weight and are difficult to dissolve, so the higher the Stiasny number is, the lower the content of other substances (Guangcheng et al. 1991; Pizzi and Mital 1994). In *P. tenuifolium*, the *Stiasny* numbers of the extracts were 64, 8 and 71.2%, which are considered high values. This index can help determine whether extracts can be used as adhesives (Chupin et al. 2013). According to Yazaki and Collins (1994), a Stiasny number of at least 65% is needed for a high-quality adhesive.

The total solids content is equivalent to the total content of extractives in powder form present in the analysed samples (Medeiros et al. 2019). In *P. tenuifolium,* the observed total solid yields were 7.8 and 11.6%. Sousa et al. (2019) observed 9.8% solids in *M. caealpiniifolia.*

Phenolic content of bark extracts

The phenolic composition of the bark extracts is shown in Table 4. The total phenolic content was 111.6 GAE g^{-1} extract, the flavonoid content was mg catechin g-1 extract, and the condensed tannin content was 501.3 CE g^{-1} extract.

Table 4 Yield of total phenols, condensed tannins and flavonoids of the bark of *Protium tenuifolium*

Total phenolic content (mg GAE g^{-1} extract)	112.6
Flavonoids (mg catechin g^{-1} extract)	5.3
Condensed tannins (mg catechin g^{-1} extract)	14.0

The phenolic content was 112.6 mg GAE g^{-1}, a lower value than in other studies, such as 165.90 mg GAE g^{-1} for *Myracrodruon urundeuva* extract (Sousa et al. 2020) and 583.0 and 682.0 mg GAE g^{-1} extract for *Anadenanthera peregrina* and *Anadenanthera colubrina* (Mota *et al*. 2017).

Flavonoids are responsible for numerous functions in plants. These functions include protection against ultraviolet rays, insects, fungi, viruses and bacteria and the ability to attract pollinating animals. In addition to exhibiting these characteristics, many of these compounds also have important pharmacological properties (Santos and Rodrigues, 2017).

The flavonoid content was only 5.3 mg catechin g-^{1} extract, which is considered low when compared to bark extract from other species, such as 91.36 mg catechin g^{-1} of *Myracrodruon urundeuva* extract (Sousa et al. 2020) and 300.8 mg catechin g^{-1} of *Myrcia eximia* extract (Araujo et al. 2020).

The tannin contents in *P. tenuifolium* bark extracts were lower than those found in other Amazonian species, such as 877 mg CE g^{-1} extract in *Myrcia eximia* (Araujo *et al.* 2020) and 59.1 mg CE g^{-1} extract in *Albizia niopoides* (Carmo et al. 2016b). In *Astronium lecointei* from the Amazon, the value was 7.3 mg CE g^{-1} of extract, which is lower than that found in *P. tenuifolium* (Mota *et al*. 2021a).

The phenolic composition of the extract quantified by HPLC is shown in Table 5. Of the seven compounds present, trigonelline was the most abundant (295.60 mg 100 g^{-1} bark), followed by catechin (259.60 mg 100 g^{-1} bark) and gallic acid (173.25 mg 100 g^{-1} bark).

Table 5 Phenolic compounds of *P. tenuifolium* bark extracts identified by HPLC

Polyphenols	**mg 100 g^{-1} bark**
Trigonelline	295.60
Gallic acid	173.25
Catechin	259.28
Vanillin	16.41
p-Coumaric acid	9.35
Ferulic acid	6.76

Catechin is a compound found in plants, and due to its chemical structure, it is an excellent antioxidant (Silva et al. 2019). It should be noted that catechin is widely used in foods, cosmetics and medicines and is a natural hydrophilic antioxidant molecule (Kiliç and Yesiloglu, 2013). Catechin was also found in the bark of the Brazilian species *Stryphnodendron rotundifolium* (Sousa *et al*. 2021). Catechin is a polyphenolic compound found in several medicinal plants (Bhadange et al. 2022).

Trigonellin has the ability to reduce glucose and has beneficial nutritional effects because it has an effect on the central nervous system and intestinal mobility (Mathur and Kamal, 2012). Gallic acid a well-known phenolic compound found in many natural sources; it is widely used in the pharmaceutical industry and has been shown to be effective in inhibiting cardiovascular diseases, such as Alzheimer's and Parkinson's diseases (Rafie et al. 2018). The phenolic compound gallic acid, which was found in the bark of *P. tenuifolium*, has higher antioxidant activity than other polyphenols (Badhani *et al.* 2015). Gallic acid was also found in the bark of the Amazon species *Myrcia eximia*.

Vanillin is another compound found in the bark of *P. tenuifolium* that is used in large quantities in drug production due to its antioxidant, antimicrobial, anti-inflammatory, analgesic and anticancer properties (Banerjee and Chattopadhyay, 2018). Vanillin is also an important raw material used in the production of drugs such as dopamine, L-dopa and aldomet (Converti et al. 2010).

p-Coumaric acid is a phytochemical with multiple human health benefits and was also found in the bark of *P. tenuifolium* in this study. p-Coumaric acid has biological activities, such as antimicrobial and anticancer activities, in addition to having many physiological functions and is considered one of the most important phenolic acids (Boz, 2015).

Ferulic acid, which was found in the bark of the species studied, is a bioactive substance with multiple functions, including antioxidant properties, anti-inflammatory properties, and the ability to remove excess free radicals and free radical-producing enzymes to reduce the damage caused by free radicals and inflammatory reactions; moreover, this compound has a protective effect against kidney and cardiovascular diseases (Li et al. 2021).

Antioxidant activity of bark extract

At almost all concentrations analysed, the bark extract of *P. tenuifolium* showed greater antioxidant activity than BHT, which was used as a standard, except at the concentration of 200 μg mL^{-1}, at which BHT inhibited 0.1% more than the bark extract. At a concentration of 25 μg mL^{-1}, the bark extract reached 83.5% inhibition against the DPPH radical, while BHT inhibited only 75.0% (Fig. 5).

At almost all concentrations, the bark extract of *P. tenuifolium* had higher antioxidant activity than BHT. The IC50 value (11.00 μg extract mL^{-1}) of *P. tenuifolium* was higher than that of BHT (IC50 = 5.69 μg extract mL^{-1}). This result indicates that a greater amount of bark extract is needed to inhibit 50% of DPPH free radicals in solution. It should be noted that at higher concentrations, the bark extract was able to

inhibit more than 90% of the DPPH free radical: concentrations of 200 and 500 μg mL^{-1} corresponded to 90.2 and 90.0% inhibition, respectively, and BHT inhibited 88.9% and 88.9%.

Branches of the species *Protium spruceanum* showed antioxidant activity (Amparo et al. 2021). The leaves of *Protium heptaphyllum* also have antioxidant activity (Patias *et al*. 2021). The essential oil of *Protium heptaphyllum* at a concentration of 1.0 mg/mL reached 83.2% inhibition of the free radical DPPH and is indicated for use in traditional drugs (Bandeira *et al*. 2005). Menezes Filho et al. (2022) reported that the essential oils of *Protium ovatum* petioles are promising due to their proven antioxidant and antifungal activity against strains of the yeast genus *Candida*, demonstrating that several species of the genus *Protium* have chemical compounds with antioxidant activity.

Conclusion

The chemical and anatomical composition of the bark of *Protium tenuifolium* was described for the first time. This species has a rhytidome, phloem, nonconductive phloem, crystals and secretory cells.

The most striking chemical characteristics of this species are its high content of polar extractives rich in phenolic compounds and high lignin content, which can be used for products that benefit from high amounts of lignin. The species also showed good antioxidant activity. Thus, the species has the potential to be used for medicinal purposes and to prepare cosmetic products, and the bark of this Amazon species has a certain value.

Declarations

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Figures

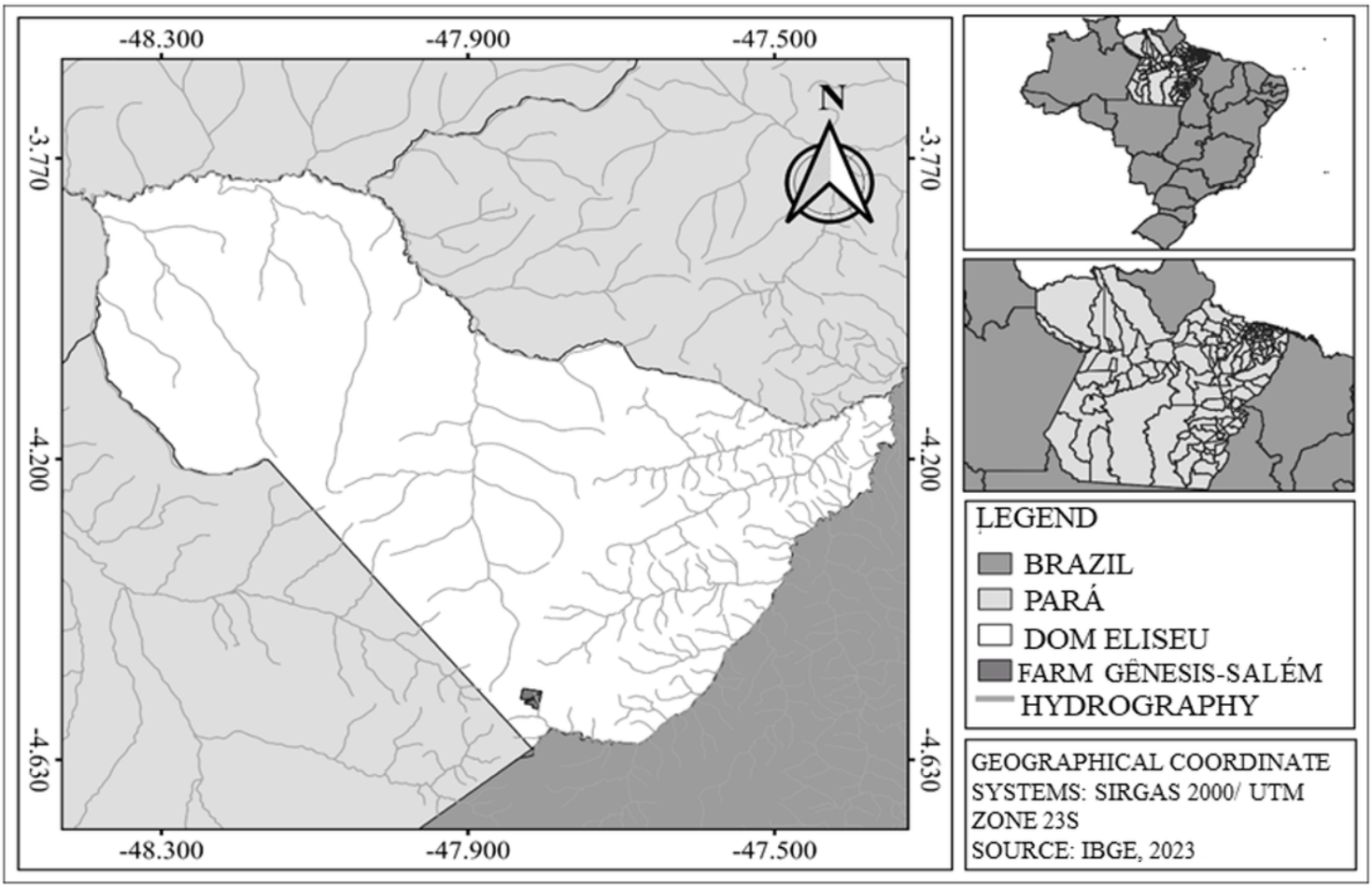

Figure 1

Location of the collection area.

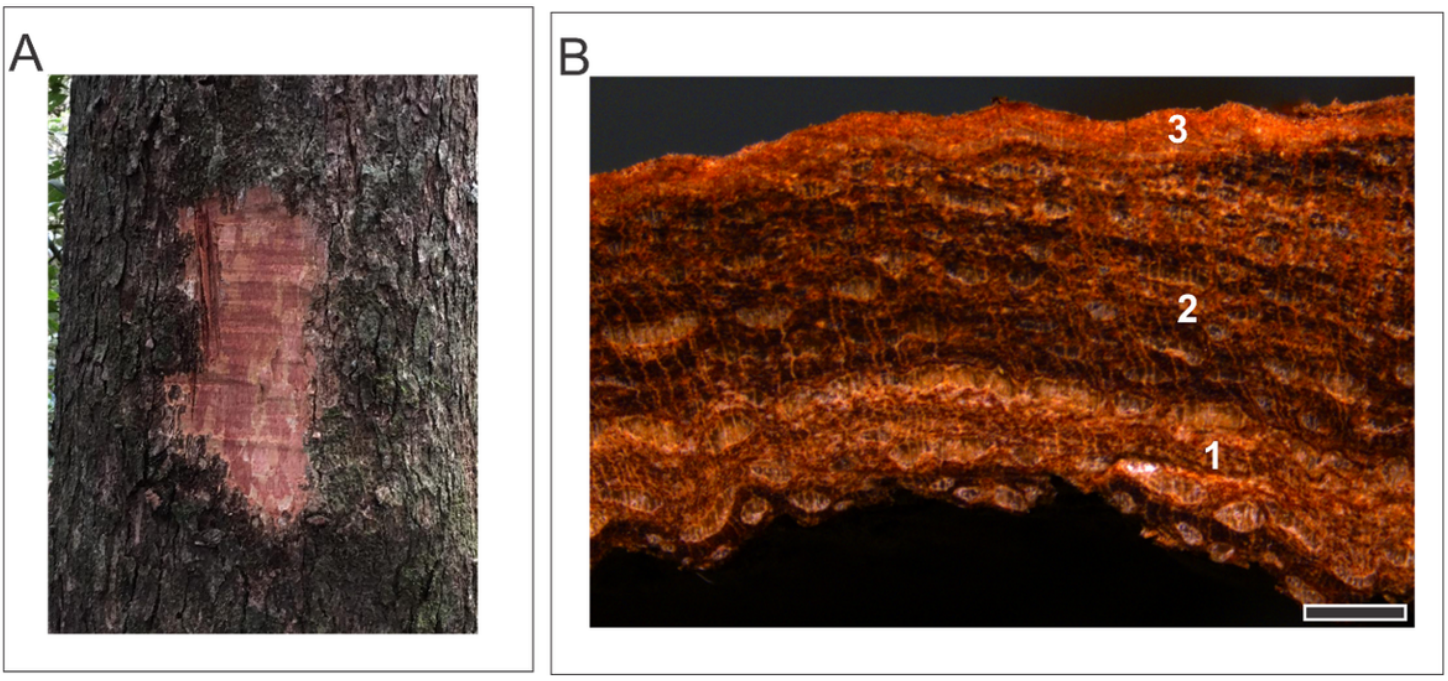

Figure 2

A Macroscopic aspects of the outer bark of *Protium tenuifolium* (Engl.) Engl. **B** Cross section of *P. tenuifolium* bark showing the conductive phloem (1), nonconductive phloem (2) and rhytidome (3). Scale bar = 100 µm.

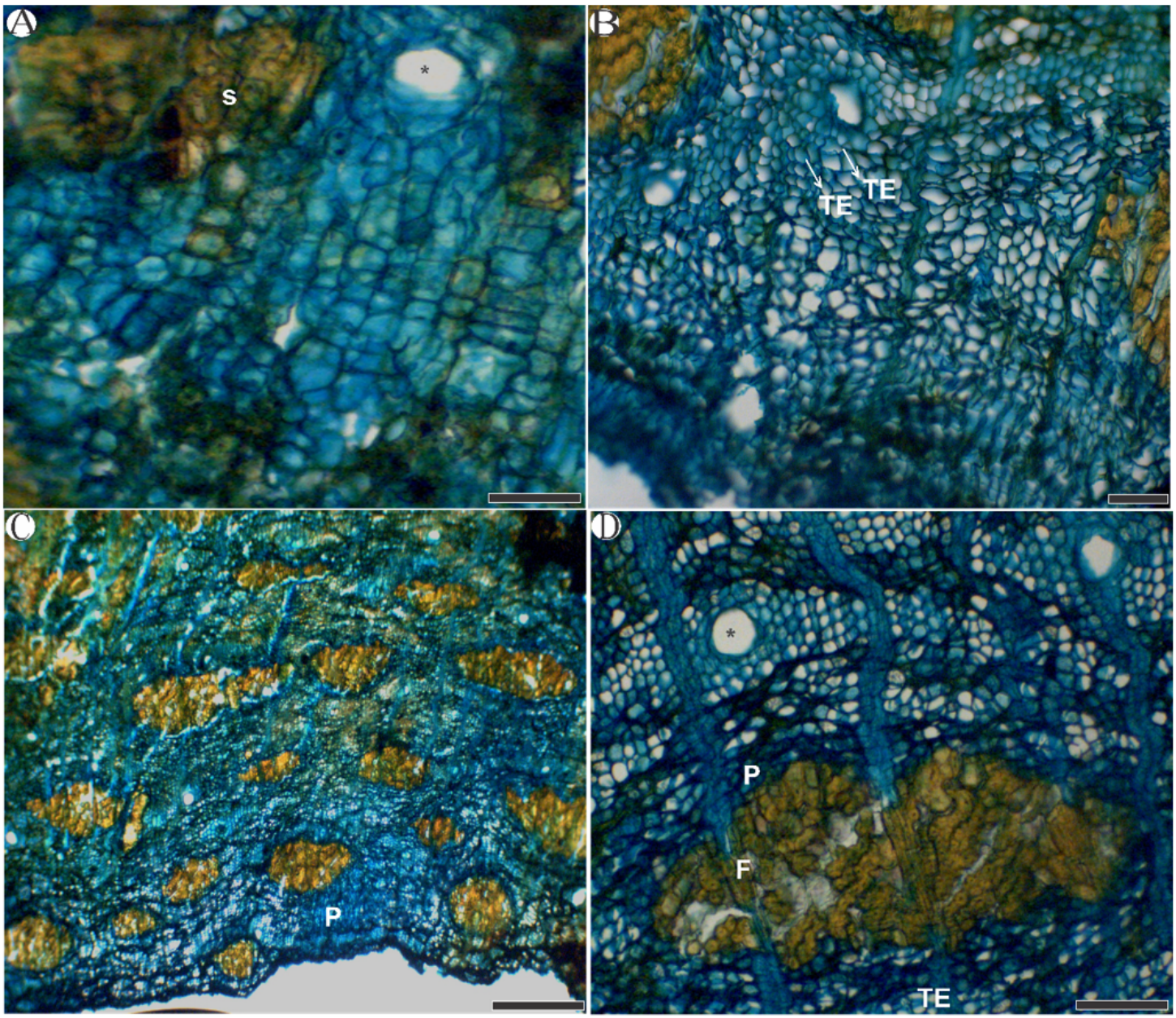

Figure 3

The transverse plane of *Protium tenuifolium*: sclereid (S) axial parenchyma cells; **B** tube element (TE), secretory cell (*); **C** phloem (P); **D** fibre (F), parenchyma (P), tube element (TE), secretory cell (*). Scale bar = 100 µm.

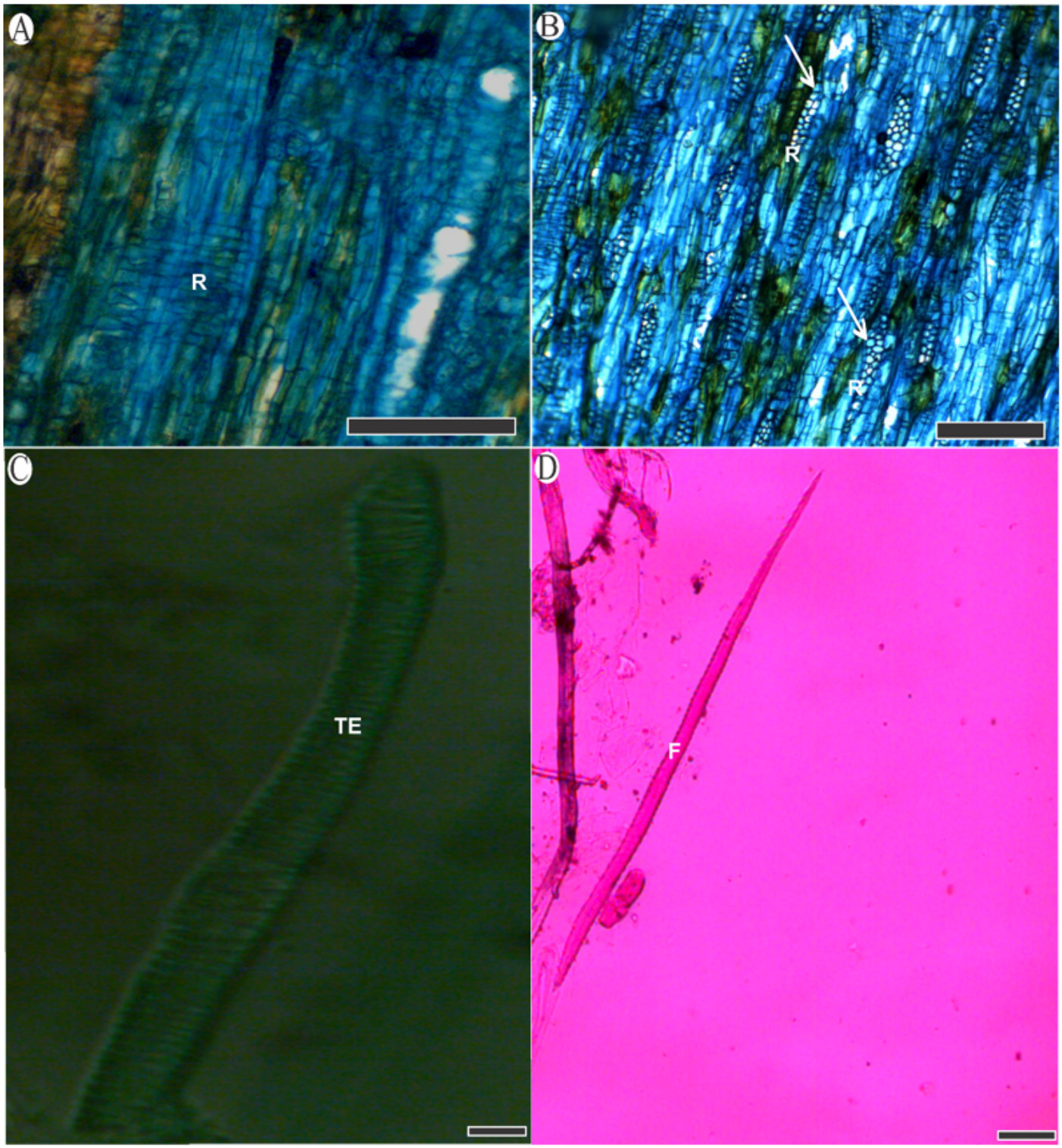

Figure 4

A Radial plane of *Protium tenuifolium*: ray (R); **B** tangential plane: ray (R), crystals (arrows); C tube element (TE): **D** fibre (F). Scale bars (A-D) = 100 µm.

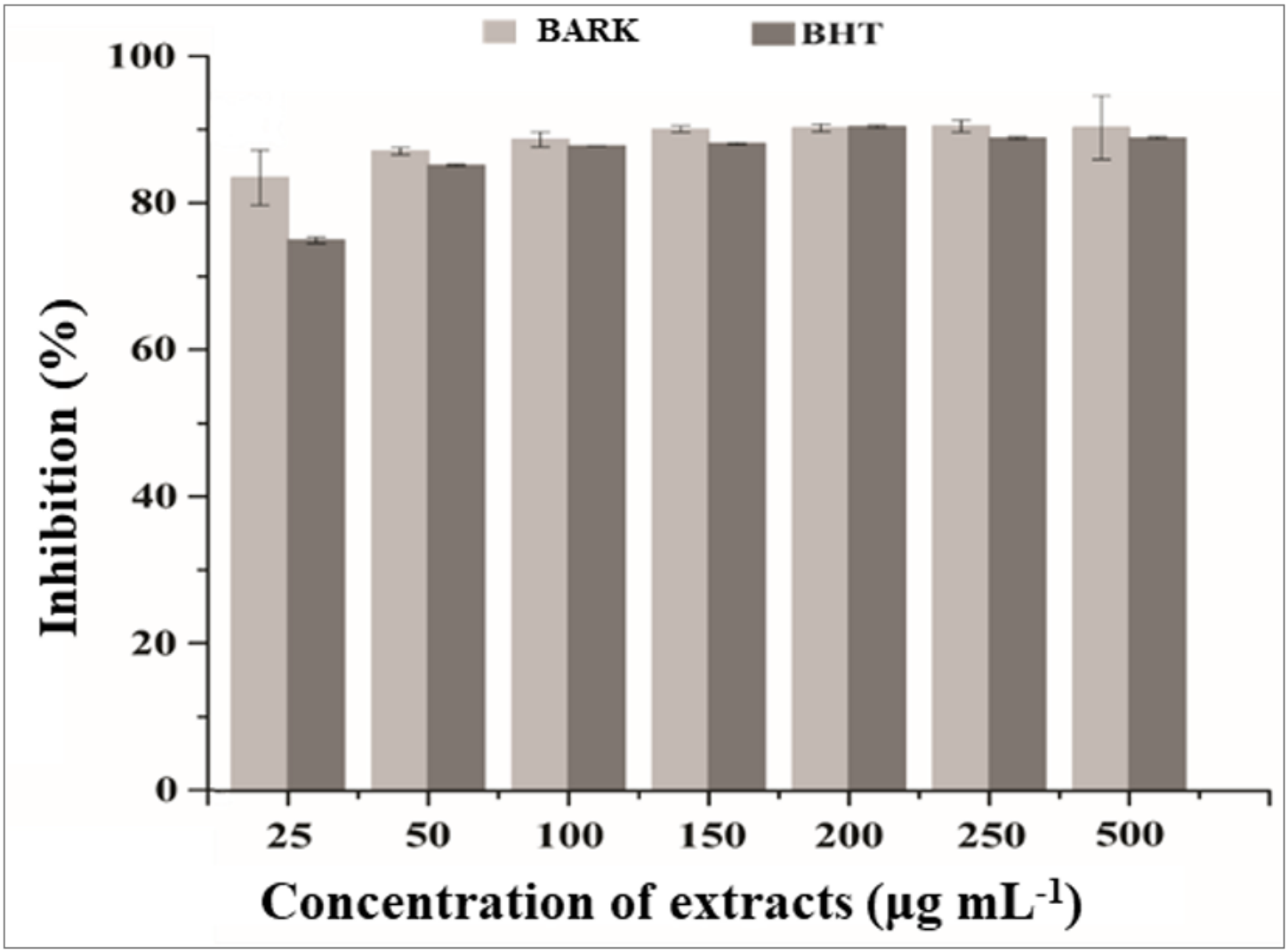

Figure 5

DPPH free radical stabilization capacity with *Protium tenuifolium* bark extract and butylated hydroxytoluene (BHT), used as a control. Each value is expressed as the mean.