

Evaluation of the antifungal potential of Fabaceae bark extracts against wood decay fungi

Evaluación del potencial antifúngico de los extractos de corteza de Fabaceae contra hongos de pudrición de la madera

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Abstract

The Amazon rainforest harbors diverse bioactive compounds with untapped potential for sustainable applications. This study evaluated the antifungal activity of bark extracts from eight Fabaceae species against wood decay fungi (*Lenzites trabea*, *Pycnoporus sanguineus*, and *Trametes villosa*). Hydroalcoholic extracts were tested in vitro to determine the antifungal index (AFI), and the chemical composition of bark and extractives was analyzed. The extracts presented fungistatic activity, with AFI values ranging from 75.05 % to 100 % at 0.10 % concentration. *Mora paraensis* (Pracuúba) demonstrated broad-spectrum antifungal activity at all tested concentrations (0.10 %, 1.00 %, and 2.00 %). In contrast, *Pentaclethra macroloba* (Paracaxi) exhibited limited efficacy, selectively inhibiting *P. sanguineus* and *L. trabea*. Chemical analysis identified tannins, flavonoids, terpenes, alkaloids, and anthraquinones as key constituents, suggesting their role in fungal inhibition. Phenolic and nitrogenous compounds correlated strongly with antifungal activity, highlighting their potential as natural fungicides. The findings support the use of Fabaceae bark extractives as sustainable alternatives to synthetic wood preservatives, promoting the sustainable utilization of forest resources. Future research should focus on optimizing extraction methods and elucidating the synergistic interactions among bioactive compounds to enhance antifungal efficacy.

Keywords: Antifungal activity, natural fungicides, wood preservation, phenolic compounds, Amazon rainforest.

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Resumen

La selva amazónica alberga compuestos bioactivos diversos con un potencial no explotado para aplicaciones sostenibles. Este estudio evaluó la actividad antifúngica de extractos de corteza de ocho especies de Fabaceae contra hongos causantes de la descomposición de la madera (*Lenzites trabea*, *Pycnoporus sanguineus* y *Trametes villosa*). Se probaron extractos hidroalcohólicos in vitro para determinar el índice antifúngico (IAF), y se analizó la composición química de la corteza y los extractivos. Los extractos presentaron actividad fungistática, con valores de IAF que variaron del 75.05 % al 100 % a una concentración del 0.10 %. *Mora paraensis* (Pracuúba) demostró actividad antifúngica de amplio espectro en todas las concentraciones probadas (0.10 %, 1.00 % y 2.00 %). En contraste, *Pentaclethra macroloba* (Paracaxi) mostró una eficacia limitada, inhibiendo selectivamente *P. sanguineus* y *L. trabea*. El análisis químico identificó taninos, flavonoides, terpenos, alcaloides y antraquinonas como componentes clave, sugiriendo su papel en la inhibición de hongos. Los compuestos fenólicos y nitrogenados se correlacionaron fuertemente con la actividad antifúngica, destacando su potencial como fungicidas naturales. Los hallazgos respaldan el uso de extractivos de corteza de Fabaceae como alternativas ecológicas a los preservantes sintéticos de madera, promoviendo la utilización sostenible de los recursos forestales. Investigaciones futuras deberían centrarse en optimizar los métodos de extracción y esclarecer las interacciones sinérgicas entre los compuestos bioactivos para mejorar la eficacia antifúngica.

Palabras clave: Actividad antifúngica, fungicidas naturales, preservación de la madera, compuestos fenólicos, selva amazónica.

Introducción

Tropical forests are among the most biodiverse ecosystems on the planet, hosting an unparalleled variety of plant species. Effective forest management practices, aligned with sustainable development principles and supported by robust legislation and oversight, are fundamental in promoting the sustainable utilization of these species. These practices not only enhance the economic value of forest products but also play a vital role in conserving biodiversity, mitigating the effects of deforestation and climate change, and maximizing social and economic benefits while maintaining the resilience of this unique ecosystem [1], [2], [3].

Amazon forest species are known to produce bioactive compounds with remarkable efficacy against insects, bacteria, fungi, and viruses. The Fabaceae family, widely distributed across Amazonian ecosystems and tropical

forests, is particularly rich in secondary metabolites such as flavonoids, terpenes, tannins, and alkaloids. Despite their promising bioactivity and economic potential, many species remain underutilized in timber production, agroforestry systems, and the extraction of natural products [4], [5].

Wood, due to its carbohydrate-rich composition, is susceptible to degradation by fungi and insects, with environmental factors such as elevated temperature and humidity further accelerating decay. However, certain timber species exhibit intrinsic resistance to these processes, due to their chemical composition and the presence of bioactive extractives. These natural extractives, which are secondary metabolites found in wood and bark, have been identified as key contributors to wood durability, influencing properties such as color, odor, and resistance to decay [6], [7], [8].

Recent advances in phytochemical research, supported by techniques such as chromatography and spectroscopy, have provided insights into the molecular structures and biological activities of these compounds [9], [10]. Laboratory studies have demonstrated their potential as environmentally friendly fungicides, inhibiting fungal growth and enzymatic activity, thereby reducing wood decay. The exploration of synergistic effects among different extractive compounds has further revealed their potential for enhanced antifungal properties, offering sustainable alternatives to synthetic wood preservatives [11], [12], [13].

Wood decay fungi play a vital role in lignocellulosic decomposition, contributing to nutrient cycling but also compromising timber durability. This study investigated the fungicidal activity of bark extractives from eight Fabaceae tree species against Fr., *Trametes villosa* (Fr.) Ryv., and *Pycnoporus sanguineus* (L.: Fr.) Murr.), well-known representatives of brown and white rot fungi. While brown rot fungi like *L. trabea* primarily degrade cellulose and hemicellulose, white rot fungi such as *T. villosa* and *P. sanguineus* break down all wood components, including lignin. The hypothesis driving this research is that these extractives possess significant antifungal properties, contributing to the resistance of lignocellulosic material. By identifying and evaluating bioactive compounds, this study aims to support the development of sustainable wood preservation strategies.

Materiales y métodos

Extractives obtained

The barks of Fabaceae trees (Table 1) were collected from the Anavilhanas Ecological Station (Amazonas, Brazil: latitude 2°00' to 3°02' S and longitude 60°27'

to 61°07' W). Extractives were obtained from sawdust through successive extractions with cold ethanol (PA grade) in Mariotti flasks. The ethanol was then evaporated under vacuum using a rotary evaporator at 50 °C. The resulting ethanol extract was placed in an oven at 60 °C, where it was macerated and weighed. Solubility tests were conducted with the extracts in water, water-ethanol, and ethanol [14], [15]. Hydroalcoholic solutions of the extractives (0.10, 1.00, and 2.0 %) were prepared and subsequently used in the bioassay.

Bioassay – antifungal activity in solid growth medium

Cultures of the fungi *Pycnoporus sanguineus* (L.: Fr.) Murr. (LPM 408) and *Trametes villosa* (Fr.) Ryv. (LPM 406), which cause white rot, as well as *Lenzites trabea* Pers.: Fr. (LPM 368), which causes brown rot, were obtained from the Lignocellulosic Fungi Collection at the National Institute for Amazonian Research (INPA/Brazil).

A sample of low-durability wood, *Simarouba amara* Aubl. (Marupá), measuring 3 × 28 × 32 mm, was placed on a culture plate. The fungus was then inoculated near the wood. The plates were maintained at 28 °C with a

relative humidity of 70 % until the fungus had colonized the wood.

An inoculum from this colony was transferred to a malt-extract agar medium. Once the fungus had grown, an inoculum ($\Phi = 4$ mm) with a vigorous mycelial appearance was selected to ensure the reliability of the tolerance and sensitivity data.

From the extractive solution, 1 mL aliquots were added to 9 mL of malt agar medium, which was then homogenized, mixed, and poured into a Petri dish. After the medium solidified, four fungal inoculums were evenly distributed on the Petri dish. The positive control included the preservative CCA (1 %), as well as ethanol (100 %) and ethanol-water mixtures (33 % and 50 %), to assess whether the solvent enhanced the antifungal potential of the extract, given that ethanol is lethal to fungi. A total of 87 Petri dish were prepared simultaneously and incubated at 28 °C with 70 % relative humidity.

The growth of the radial mycelium was measured in millimeters in both linear and vertical directions using a Mitutoyo MyCAL6CS digital caliper. Measurements

Table 1. Fabaceae species selected for the study.

Tabla 1. Especies de Fabaceae seleccionadas para el estudio.

Scientific name/Popular	No. INPA Library	General characteristics [16]
<i>Albizia subdimiata</i> (Splitg.) Barneby & J.W.Grimes/ Faveira-do-igapó	X-10.116	Evergreen tree frequently found near stagnant water bodies, found in Bolivia, Brazil (Amazonas), Colombia, Guyana, Peru, and Suriname.
<i>Aldina heterophylla</i> Spruce ex Benth./ Macucu-de-paca	X-10.127	Medium to large trees found in areas of campinarana and sandy soil. This species is endemic to Brazil (Amazonas) and is economically important due to the value of its timber.
<i>Crudia amazonica</i> Spruce ex Benth/ Lombrigueiro	X-10.119	Trees of medium size occur on the sandy banks of rivers and igapós. It occurs in the Brazilian Amazon, and in French Guiana and Colombia.
<i>Macrolobium angustifolium</i> (Benth.) R.S.Cowan/ Sucupira-preta	X-10.123	Medium-sized tree, frequently found in flooded areas. It is a neotropical species found in Brazil (Amazonas), Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, and Venezuela.
<i>Mora paraensis</i> (Ducke) Ducke/ Pracuúba	X-9.598	Large tree with thick trunk, found in various places along the banks of the Rio Negro (Amazonas/Brazil). Native to South America, it is found in Colombia, French Guiana, Peru, and Suriname.
<i>Pentaclethra macroloba</i> (Willd.) Kuntze/ Paracaxi	X-10.200	Tree of medium size, common in igapós and occasionally on terra-firme, found in Brazil, Colombia, Costa Rica, French Guiana, Nicaragua, Panama, Peru, and Suriname.
<i>Stryphnodendron guianense</i> (Aubl.) Benth./ Faveira-camuzé	H-200.709	Trees of medium size, occurring in ancient secondary forests on sandy soils, and margins of savannah forests. Found in Bolivia, Brazil, Peru, Suriname, and Venezuela.
<i>Tachigali paniculata</i> Aubl./Tachi-preto	H-200.688	It is common in the floodplains and igapós of rivers with poor water in sediment, less frequent in the forest and in the capoeira of the terra-firme, clayey. It occurs in Brazil, Bolivia, Peru, and the Guianas.

were taken every 3 days until the 12th day, the required period for fungal development.

The area of the fungal colony (cm²) was calculated based on the average radial mycelium growth (mm) from four replicates for each extractive solution concentration and solvent. The Antifungicidal Index (AFI) was determined based on the fungal colony area (cm²) on the twelfth day of incubation and was expressed as a percentage using the equation described by Jesus et al. [11].

Determination of the constituents of bark of Fabaceae

For a complete characterization of the material, the chemical constituents and phytochemical classes of the bark extracts were evaluated. The bark obtained from at least three trees (per species) was processed in a knife mill to obtain sawdust in 60 mesh, and the following analyses were performed in triplicate.

Total extractives - ethanol/toluene

A 2.00 g aliquot of sawdust was extracted with ethanol/toluene for approximately 8 hours via a Soxhlet apparatus. Subsequently, ultrasonic extraction with water was performed at 65 °C for 4 hours. The total extractives content (TE %) was calculated as follow [17] (equation 2).

$$TE \% = \Sigma mass/Wd * 100 \quad (2)$$

Where: $\Sigma mass$ = total mass of extracted substances. Wd = weight of the dry sample.

Solubility in hot water

Sawdust extracted with ethanol-toluene was dried, weighed, and transferred to an Erlenmeyer flask. Boiling distilled water was added and heated in a water bath for 4 hours, with the water being changed hourly. The material was filtered, dried at 100 °C for 24 hours, and weighed to determine soluble material content [17] (equation 3).

$$Hot\ water\ \% = (P1-P2)/Wd * 100 \quad (3)$$

Where: P1 is weight of the initial sample; P2 is sample weight after extraction; Wd is weight of the dry sample.

Klason lignin

A 1.00 g sample of extractive-free wood was weighed into a beaker and treated with 72 % H₂SO₄. The mixture was allowed to rest for 2 hours (in a cold bath); after which 560 mL of water was added. The beaker was then placed in a water bath (100 °C) for 4 hours. After the reaction time, the samples were filtered through a Gooch crucible (M). The material was washed with

500 mL of heated distilled water and dried in an oven (100 °C) for 12 hours. The samples were subsequently weighed to a constant weight, and the lignin content was determined [17] (equation 4).

$$Lignin\ \% = W2/W1 * 100 \quad (4)$$

Where: W1 is initial dry weight of the sample; W2 is dry weight of the obtained lignin.

Holocellulose

Holocellulose determination was performed using 1.00 g of extractive-free sawdust. The material was treated with 20 mL of 3 % HNO₃, refluxed for 30 minutes, filtered, and then treated with 25 mL of 3 % NaOH. The residue was washed with ethanol, ethanol-water solution, and water, then dried and weighed to determine cellulose + hemicellulose content [18] (equation 5).

$$Holocellulose\ \% = P2/P1 * 100 \quad (5)$$

Where: P1 is initial dry weight of the sample; P2 is dry weight of the raw cellulose obtained.

Ash

In a porcelain crucible, 1.00 g of sawdust was added, and the mixture was placed in an oven (100 ± 3 °C) for 1 hour to remove moisture. The crucible was then transferred to a muffle furnace for incineration, starting with gradual heating to 580–600 °C. After incineration, the crucible was weighed to a constant weight. The ash content was determined using the following equation 6 [17].

$$Ash\ \% = Wash/Wd * 100 \quad (6)$$

Where: Wash is weight of the ash; Wd is weight of the dry sawdust.

Phytochemical classes

Extractives obtained from the bark (see description above) were tested according to Varejão et al. [4] and Simões et al. [19].

Terpenoids

Steroids and Triterpenes - In a beaker, 10 mL of the extract was evaporated to dryness using a water bath. The dried material was then extracted with three portions of 2 mL chloroform each. The extract was filtered using a funnel with cotton and a small amount of anhydrous Na₂SO₄ into a thoroughly dried test tube. Next, 1 mL of acetic anhydride was added, followed by gentle stirring, and three drops of concentrated H₄SO₄. The mixture was stirred again, and color changes were observed. A transient blue color followed by a stable green indicated the presence of free steroids, whereas

a brownish-red coloration suggested the presence of triterpenoids. *Brosimum rubescens* extract was used as the standard for steroids, and *Buchenavia parviflora* extract for triterpenes; Saponins - A total of 2 mL of hydroalcoholic extract was dissolved in 2 mL of water in a test tube. The tube was vigorously shaken for 2–3 minutes, and foam formation was observed. The presence of persistent and abundant foam indicated the presence of saponins.

Nitrogen Compounds

Alkaloids - A total of 5 mL of extract was concentrated in a water bath to half its original volume, adjusted to pH 4, and filtered. The solution was then alkalized to pH 11 by adding NH_4OH and extracted with successive portions (30, 20, and 10 mL) of an ether-chloroform mixture (3:1) using a separatory funnel. The organic phase was dried using anhydrous Na_2SO_4 to remove excess water. The aqueous phase was then removed and divided into three test tubes. Three drops of alkaloid precipitation reagents (Hager, Mayer, and Dragendorff) were added to each tube. The presence of alkaloids was confirmed by the formation of a flocculent precipitate in at least two tubes. Aniba roseodora extract was used as a standard for alkaloids; **Cyanogenic Heterosides** - A total of 10.00 g of sample (sawdust) was mixed with 50 mL of water and 1 mL of 5N H_2SO_4 in a 250 mL Erlenmeyer flask with a lid. A strip of sodium picrate paper was attached to the lid. The paper strips (7 cm × 1 cm) were first immersed in a solution of Hager's reagent, dried, then immersed in a 10 % Na_2CO_3 solution, and dried again before use. The strip was positioned without direct contact with the liquid.

Phenolic Compounds

Phenols and Tannins - In a test tube, 2 mL of extract was mixed with three drops of an alcoholic FeCl_3 solution, followed by stirring, and any color variation or precipitate formation was observed. Additionally, 2 mL of neutral lead acetate was added to compare different types of tannins. These reactions were evaluated against a blank test using water and FeCl_3 , as well as reference compounds: catechin (for condensed tannins) and gallic acid (for hydrolyzable tannins). A color change ranging from blue to red indicated the presence of phenols, with blue suggesting hydrolyzable tannins and green indicating condensed tannins. The presence of hydrolyzable tannins was further confirmed by the formation of a flocculent precipitate (white salt) with a bluish hue upon the addition of lead acetate; **Flavonoids** - A total of 2 mL of extract was added to three separate test tubes. The pH of each tube was adjusted as follows: one to pH 3 (acidic), another to pH 8.5 (neutral), and the third to pH 11 (alkaline). The appearance of different colors was observed, indicating the presence of various flavonoid constituents; **Anthraquinones and Anthranols**

- In a test tube, 5 mL of an ethereal solution was mixed with 2 mL of 6N NH_4OH solution. The mixture was stirred thoroughly until two distinct phases formed. A red coloration in the aqueous layer indicated the presence of hydroxylated anthraquinones. If the previous test was negative, 1 mL of hydrogen peroxide was added to the same tube, stirred, and left undisturbed until phase separation occurred. After 10 minutes, the appearance of a red color in the aqueous phase indicated the presence of anthranols. *Senna* sp. extract was used as a standard for anthraquinones.

Data analysis

All statistical analyses were conducted using OriginPro 2024b. Analysis of variance (ANOVA) was performed to assess differences in AFI and chemical composition. When significant effects were detected ($p < 0.05$), Tukey's post-hoc test were applied for multiple comparisons. Additionally, principal component analysis (PCA) was used to explore the relationships between extract composition and antifungal activity. PCA was conducted on standardized data, and loadings and score plots were generated to identify key chemical constituents associated with antifungal effects.

Resultados y discusión

The antifungal activity of hydroalcoholic extracts from the bark of species in the Fabaceae family was evaluated against three wood decay fungi (*Lenzites trabea*, *Pycnoporus sanguineus*, and *Trametes villosa*) to identify bioactive compounds and assess their potential as natural fungicides. The results showed significant variation in antifungal efficacy among the extracts, depending on the species, concentration, and type of fungus (Table 2).

The extractive solutions presented fungistatic activity, with an antifungal index (IAF) ranging from 75.05 % to 100 % at concentrations between 0.10 % and 2.00 % for all fungi, except for the extracts of *Pentaclethra maculoba* (Paracaxi), which did not present fungitoxic activity against *T. villosa*. The extracts with the lowest concentration (0.10 %) and effectiveness were observed in *Aldina heterophylla* (Macucu-de-paca), *Crudia amazonica* (Lombrigueiro), *Stryphnodendron guianense* (Faveira-camuzé), and *Tachigali paniculata* (Tachi-preto). At the intermediate concentration (1.00 %), extracts of *Albizia subdimidiata* (Faveira-do-igapó) demonstrated consistently high IAF values (94.36 % to 99.77 %). Notably, extracts of *Mora paraensis* (Pracuúba) were effective at all evaluated concentrations (0.10 %, 1.00 %, and 2.00 %) against all fungi, highlighting their broad-spectrum antifungal activity (Figure 1 and 2). In contrast, *P. maculoba* extracts showed limited efficacy,

Table 2. Antifungal index of bark extracts of the Central Amazonian Fabaceae against wood decay fungi.
Tabla 2. Índice antifúngico de extractos de corteza de las Fabaceae la Amazonía Central contra los hongos de descomposición de la madera.

Fungus	Concentration (%)	<i>Albizia subdimidiata</i>	<i>Aldina heterophylla</i>	<i>Crudia amazonica</i>	<i>Macrobium angustifolium</i>	<i>Mora paraensis</i>	<i>Pentaclethra macroloba</i>	<i>Stryphnodendron guianense</i>	<i>Tachigali paniculata</i>	Positive control (CCA)	Water-ethanol 50 %	Water-ethanol 33 %	Ethanol 100 %	Negative (0.00 %)
<i>Lenzites trabea</i>	0.1	30.49c (±31.18)	86.75b (±3.61)	96.11a (±0.08)	29.03c (±17.11)	94.49a (±6.18)	30.87c (±16.00)	93.86a (±9.98)	93.15a (±5.01)	100.00a	0.00d	0.00d	100.00a	0.00d
	1	99.77a (±1.08)	67.50c (±8.48)	70.68bc (±1.08)	85.14b (±4.21)	97.48a (±1.31)	91.51a (±1.21)	79.58b (±11.18)	94.91a (±0.91)	100.00a	0.00d	0.00d	100.00a	0.00d
	2	79.84b (±1.35)	63.67c (±9.10)	94.61a (±13.51)	80.79b (±5.08)	97.69a (±1.10)	92.80ab (±1.51)	91.15ab (±8.22)	69.97bc (±1.99)	100.00a	0.00d	0.00d	100.00a	0.00d
<i>Pycnoporus sanguineus</i>	0.1	61.27c (±7.60)	100.00a	97.39a (±1.33)	98.09a (±1.18)	100.00a	81.27b (±5.20)	100.00a	100.00a	100.00a	0.00d	0.00d	100.00a	0.00d
	1	96.21a (±3.93)	41.52c (±21.11)	53.07bc (±3.93)	52.83bc (±6.18)	94.36a (±2.16)	42.56c (±14.01)	45.81c (±5.18)	57.30bc (±3.93)	100.00a	0.00d	0.00d	100.00a	0.00d
	2	57.30c (±11.11)	0.00d	0.00d	0.00d	86.69ab (±4.77)	63.28bc (±9.22)	51.54bc (±7.01)	90.94a (±3.44)	100.00a	0.00d	0.00d	100.00a	0.00d
<i>Trametes villosa</i>	0.1	39.04cd (±8.21)	85.80b (±1.18)	75.18b (±9.13)	94.47a (±2.10)	100.00a	68.88bc (±9.91)	89.14ab (±5.18)	75.05b (±6.08)	100.00a	0.00e	0.00e	100.00a	0.00e
	1	94.68a (±4.01)	42.10cd (±13.18)	77.84b (±3.87)	52.07c (±8.18)	94.30a (±1.96)	53.16c (±13.20)	47.81cd (±13.11)	45.98cd (±3.87)	100.00a	0.00e	0.00e	100.00a	0.00e
	2	30.20cd (±9.19)	0.00e	0.00e	0.00e	92.02a (±5.41)	61.76b (±9.91)	40.14c (±9.14)	93.27a (±2.13)	100.00a	0.00e	0.00e	100.00a	0.00e

Means followed by the same letter (line) do not differ by Tukey's test ($p \leq 0.05$); in parentheses, standard deviation

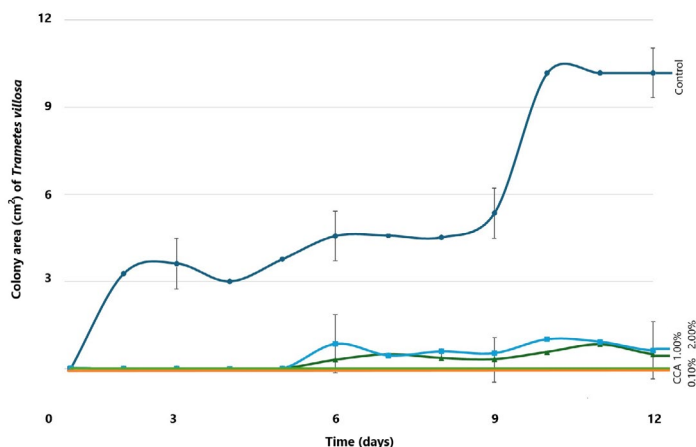


Figure 1. Effect of the bark extractive solutions of the species *Mora paraensis* on the *Trametes villosa*

Figura 1. Efecto de las soluciones extractivas de corteza de la especie *Mora paraensis* sobre la *Trametes villosa*.

selectively inhibiting only *L. trabea* (at concentrations of 1.00 % and 2.00 %) and *P. sanguineus* (at 0.10 %). These results corroborate the findings of Jesus et al. [11], Barbero-López [20], Vettraino et al. [21], and Vovchuk et al. [22], who also observed the antifungal efficacy of plant extracts against wood decay fungi. The observed differences in activity suggest that the chemical composition of the bark extractives plays a fundamental role in their antifungal potential.

The solvent used to extract the extractives significantly influenced the antifungal properties of the solutions. While ethanol/water mixtures (33 % and 50 %) presented no inhibitory effects, pure ethanol (100 %) demonstrated complete inhibition of fungal growth, achieving an AFI of 100 % (Table 2). This result highlights the importance of solvent polarity in the extraction of bioactive compounds, with pure ethanol being more effective in isolating compounds with antifungal properties. The performance of ethanol was comparable to that of the synthetic biocide CCA (chromium, copper, and arsenic), which also presented an AFI of 100 % at all concentrations evaluated, a result supported by the literature [22], [23].

For a comprehensive evaluation of the composition of the studied barks, macrometabolites (holocellulose and lignin), secondary metabolites (extractives), and fixed mineral residue (ash) were quantified (Figure 3). These values fall within the expected range for barks of tropical tree species [6, 24]. Total extractives (ethanol + toluene) varied among species. *M. paraensis* and *Pentaclethra macroloba* (Paracaxi) presented the highest extractive contents (25.53 and 25.15 %, respectively). The fraction

extracted with hot water presented smaller variations among species, being slightly higher for *A. subdimidiata* and *M. angustifolium* (~15 %). According to ASTM [17] and Simões et al. [19], solvents such as ethanol + toluene can extract lipophilic and nonpolar compounds, including resins, phenolic compounds, steroids, and some alkaloids, while hot water typically removes water-soluble compounds, such as polyphenols, flavonoids, and simple sugars. The extractive compounds are responsible for the color, taste, and flavor of the lignocellulosic material, as well as for its resistance, since the bark serves as the first protective barrier of the plant against physical, chemical, and biological stresses [4], [6], [25].

The results of the chemical characterization of the bark reflect the variability among species of the Fabaceae family, highlighting differences in their potential applications. The high levels of extractives in these species underscore their relevance for the development of natural adhesives and biocides, while the high content of holocellulose can be used in the manufacture of non-structural engineered products, and the high concentration of lignin may be suitable for energy purposes. The methodologies used to extract and evaluate the fungicidal activity of extractives should be carefully considered, given the diversity of compounds present in the bark tissue [26].

The phytochemical composition of the extracts is fundamental for evaluating the interaction of antifungal compounds. These systematic approaches provide valuable insights into the potential of extractives to combat wood decay fungi [21]. The chemical composition of the Fabaceae extractives studied includes tannins, flavonoids, terpenes, alkaloids, anthraquinones, and others (Figure 4). In the extracts of *C. amazonica*, *M. angustifolium*, and *M. paraensis*, a greater number of chemical classes were detected (chemical class index, CCI = 0.47), indicating a phytochemical profile composed of steroids, saponins, alkaloids, quaternary bases, xanthenes, and condensed tannins. In contrast, the lowest CCI was detected in the extracts of *S. guianense*.

Compounds such as triterpenes and saponins (terpenoids) are widely recognized for their antimicrobial properties, while nitrogenous compounds, such as alkaloids, are often associated with toxic activities against microorganisms. Phenolic compounds, a diverse category that includes tannins and flavonoids, can disrupt fungal metabolism by interfering with enzymatic activity and cellular integrity, leading to reduced growth and viability of fungi [27], [28], [29].

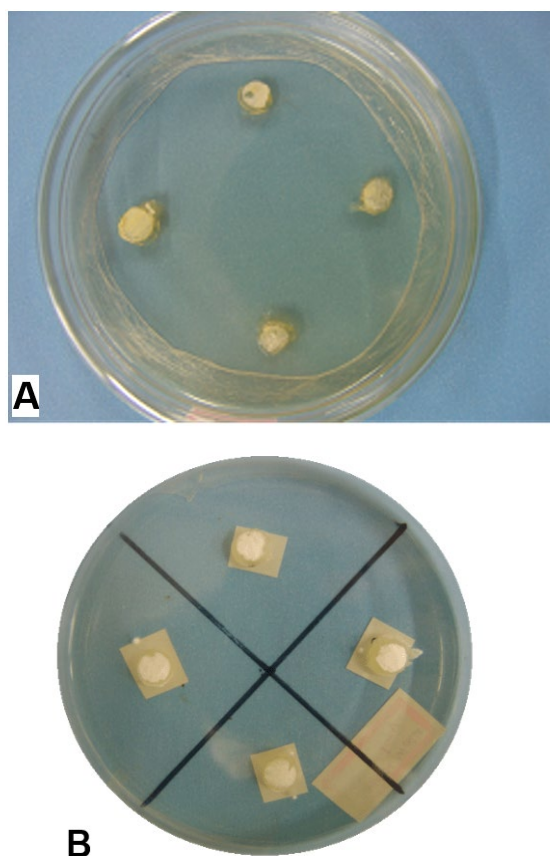


Figure 2. Bioassay the antifungal activity of extracts from the bark of *Mora paraensis* for the fungus *Trametes villosa*: A – extract 0.10 %; B – ethanol (100 %).

Figura 2. Bioensayo de la actividad antifúngica de extractos de corteza de *Mora paraensis* para el hongo *Trametes villosa*: A – extracto 0,10 %; B – etanol (100 %).

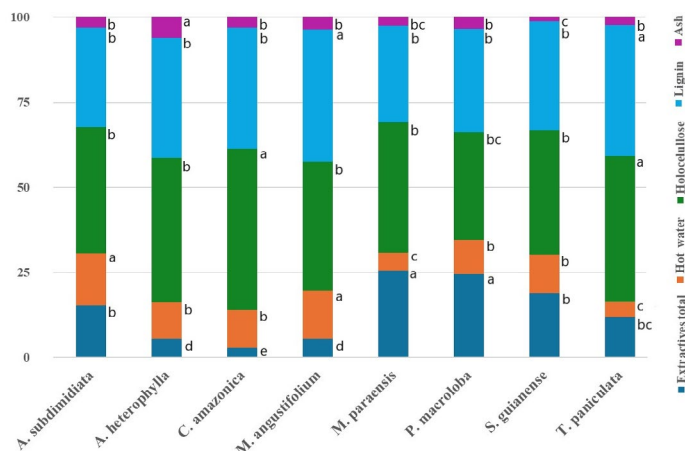


Figure 3. Chemical compounds of the bark of studied species of Fabaceae.

Figura 3. Constituyentes químicos de la corteza de las especies Fabaceae estudiadas

Interestingly, the results did not indicate a direct correlation between total extractive content (Figure 3), CCI (Figure 4), and antifungal activity (Table 2), as species with high extractive content did not always present higher AFIs. For example, *S. guianense* presented a low CCI (0.29), but its extracts were effective in exhibiting fungistatic activity. In contrast, extracts from *P. macroloba*, despite having a high extractive content (25.15 %), demonstrated moderate efficacy compared to those from other species. This suggests that specific bioactive compounds, rather than the total extractive content, are critical determinants of antifungal efficacy. Another explanation for the antifungal activity of the extracts is that the bioactive compounds were present in low concentrations or that the extracts did not preset fungistatic action against the organisms evaluated.

Pearson correlation analysis (Table 3) revealed significant associations between certain chemical classes and antifungal activity. Specifically, phenolic compounds demonstrated strong correlations with high AFI values for the three fungi: *L. trabea* ($r = 0.89$), *P. sanguineus* ($r = 0.99$), and *T. villosa* ($r = 0.94$). This finding highlights their critical role in antifungal mechanisms. A significant correlation was also observed between nitrogenous compounds and the fungi *L. trabea* ($r = 0.94$) and *P. sanguineus* ($r = 0.88$). Principal Component Analysis (PCA) further underscored the importance of specific chemical classes, with phenolic and nitrogenous compounds emerging as key determinants of antifungal efficacy (Figure 5). These results corroborate the findings of Vovchuk et al. [22] and Del Frari et al. [30], who emphasized the role of chemical synergy in enhancing bioactivity.

Research indicates that the high natural resistance of wood is attributed to the presence of various classes of chemical compounds [15], [23]. Extractives enriched with terpenoids, alkaloids, and phenolic compounds (e.g., flavonoids, tannins, and anthraquinones) contribute significantly to wood durability, highlighting their potential for biocidal applications [5], [31]. Furthermore, the capacity of bioactive compounds to disrupt fungal cell membranes and growth pathways underscores their utility in developing natural and effective wood preservatives. As antifungal resistance becomes an increasing concern, research on plant-derived extractives represents a vital step toward sustainable and environmentally friendly wood preservation techniques [32].

Conclusions

This study highlights the antifungal potential of bark extractives from Fabaceae tree species, demonstrating

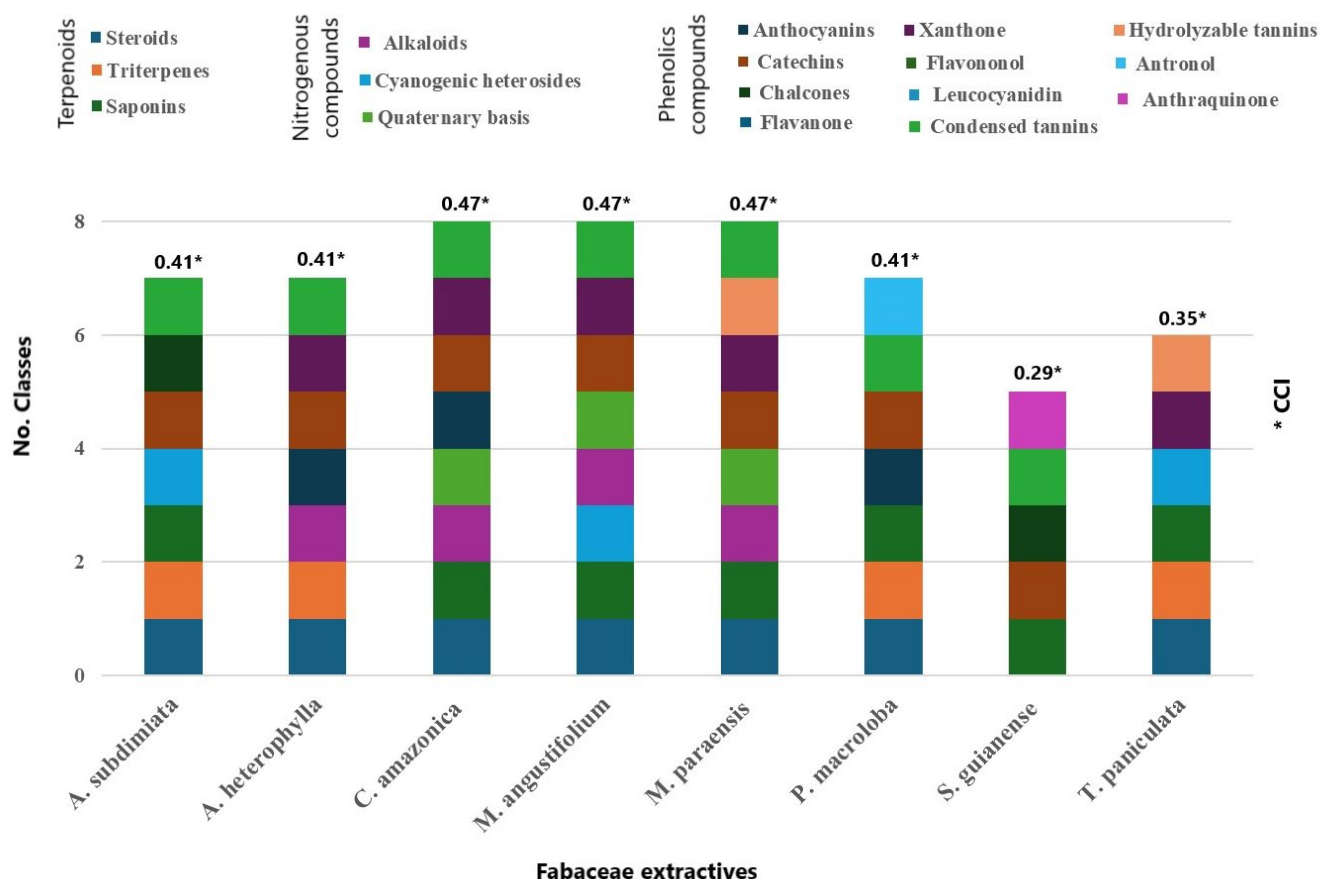
Table 3. Pearson correlation of extractive variables and AFI of wood decay fungi.**Tabla 3.** Correlación de Pearson de variables extractivas y AFI de hongos de descomposición de la madera.

r	Terpenoides	Nitrogenous compounds	Phenolics compounds	<i>Lenzites trabea</i>	<i>Pycnoporus sanguiueus</i>	<i>Trametes villosa</i>
Extractives total	0.77	0.33	0.70	0.43	0.99*	0.54
Hot water	0.58	0.79	0.84	0.85	0.33	0.30
Chemical class index	0.81	0.02	0.70	0.98*	0.56	0.40
<i>Terpenoides</i>	-	-	-	0.82	0.19	0.40
<i>Nitrogenous compounds</i>	-	-	-	0.94*	0.88**	0.26
<i>Phenolics compounds</i>	-	-	-	0.89**	0.99*	0.94**

* $p > 0.01$; ** $p > 0.05$

their viability as natural alternatives to synthetic wood preservatives. The results confirm that the extracts contain bioactive compounds with antifungal properties, particularly phenolic and nitrogenous compounds, which were strongly correlated with high antifungal indices.

Among the studied species, *Mora paraensis* and *Stryphnodendron guianense* presented the most consistent and broad-spectrum antifungal activity, effectively inhibiting all tested fungi at various concentrations. In contrast, *Pentaclethra macroloba* demonstrated limited efficacy, suggesting that the

**Figure 4.** Classes of compounds found in the barks extracts investigated.**Figura 4.** Clases de compuestos encontrados en los extractos de corteza investigados.

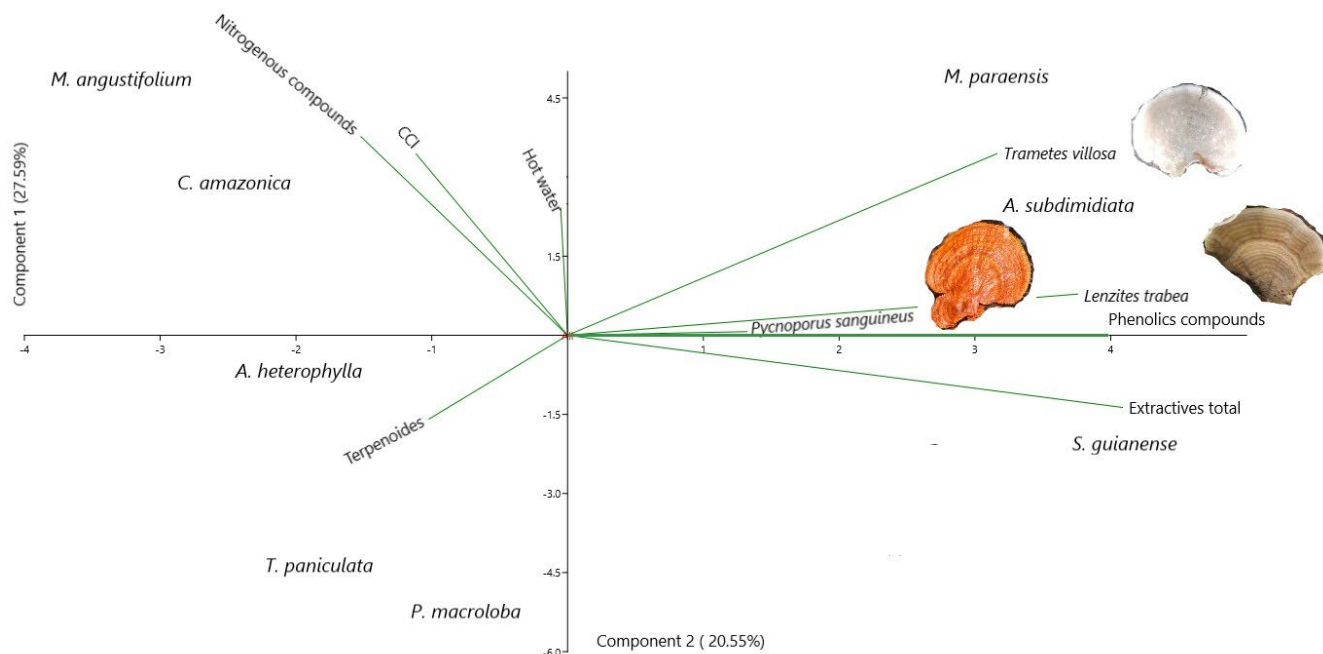


Figure 5. PCA of the studied variables.

Figura 5. PCA de las variables estudiadas.

antifungal potential is not solely dependent on total extractive content but rather on the presence of specific bioactive compounds.

The findings indicate that phenolic compounds, including tannins and flavonoids, as well as nitrogenous compounds such as alkaloids, play a key role in the inhibition of wood decay fungi. These compounds likely interfere with fungal metabolism and cell membrane integrity, leading to reduced fungal growth. However, the variation in antifungal activity among species suggests that synergistic interactions between different chemical classes may enhance or suppress the overall effectiveness of the extracts.

Future research should focus on optimizing extraction methods to maximize the yield and concentration of these bioactive compounds. Additionally, further investigation into the mechanisms of action and potential synergistic effects between different chemical classes will provide deeper insights into the antifungal properties of Fabaceae bark extractives. These findings contribute to the sustainable use of Amazonian Forest resources by promoting eco-friendly solutions for wood preservation.

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