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First report on the phytochemical composition and pharmacological activities of *Breonia perrieri* Homolle, An endemic RUBIACEAE of Madagascar

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Abstract

Breonia perrieri Homolle (RUBIACEAE) is a tree species belonging to an endemic Malagasy genus. It is currently threatened by the overexploitation of its timber, which is widely used in carpentry and construction. Apart from existing botanical information, no scientific studies have been conducted on its chemical composition or biological properties to date. This study aimed, to promote the valorization of this species through phytochemical and pharmacological investigations, as well as to disseminate the first scientific findings concerning this plant to contribute to its conservation. Phytochemical analyses of crude extracts from leaves, bark, and wood revealed a high diversity of secondary metabolites of pharmacological interest, which are characteristic of the RUBIACEAE family, and exhibit variable distribution among the different plant organs. This variability suggests metabolic specialization within the various plant tissues. Biologically, antibacterial activity, as assessed by the agar diffusion and broth microdilution methods, demonstrated selectivity effect against *Streptococcus pneumoniae*, with a minimum inhibitory concentration (MIC) of 250 µg/ml. The extracts also exhibited remarkable free radical scavenging activity in the DPPH assay, with IC_{50} values ranging from 6.56 to 99.47 µg/ml, comparable to those of the reference compounds used. These results implay that *B. perrieri* is a promising source of bioactive molecules that could be used to develop of new phytomedicines for treating bacterial infections and disorders related to oxidative stress-related. This first comprehensive study enriches our knowledge of the species and underscores the importance of implementing conservation and propagation strategies to ensure its long-term sustainability.

Keywords: *Breonia perrieri*; RUBIACEAE; Report; Phytochemical; Pharmacological

1. Introduction

Madagascar is recognized as a true biodiversity hotspot due to its exceptional biological richness. The island is home to more than 14,000 plant species, 80 % of which are endemic [1]. Paradoxically, the Malagasy population is one of the fastest growing in the world, resulting in the extensive degradation of forest resources [2].

This rapid population growth exerts considerable pressure on biodiversity through various forms of anthropogenic disturbance. These include illegal logging; unsustainable production systems adopted over several decades, agricultural practices such as *tavy* (slash-and-burn cultivation); increased demand for wood as a primary source of energy and construction material in urban areas; and bushfires, which are highly detrimental to natural ecosystems [3,4,5].

Consequently, Madagascar loses thousands of hectares of forest annually, with an estimated 20,000 to 30,000 hectares of natural forest destroyed each year. These forests harbor most of the island's plant diversity. According to Harper *et al*. (2007), Madagascar's annual deforestation rate in was approximately 0.9 % between 1990 and 2000 [6]. Such a high

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deforestation rate threatens not only Malagasy forests, but also the numerous medicinal plant species they contain. Many of these species face a real risk of extinction before their potential value can be discovered and scientifically documented [7].

Many tree species in the RUBIACEAE family in Madagascar are extensively exploited for timber and construction purposes. This is particularly true for the genus *Breonia* A.Rich. ex DC., which belongs to the Naucleeae tribe (RUBIACEAE, CINCHONOIDEAE) and is endemic to Madagascar. Members of this genus are characterized by large trees that sometimes reach up to 30 m in height or, more rarely, by shrubs. All *Breonia* species produce high-quality hardwood that is easy to work with. Consequently, their timber is widely used in construction, for boat manufacturing, and for the production of bridges, furnitures, and other building materials. Representative species include *B. boivinii*, *B. chinensis*, *B. decaryana*, *B. cuspidata*, *B. fragifera*, and *B. perrieri* [8,9].

In Madagascar, species of the genus *Breonia* occupy a wide range of habitats, including humid tropical forests in the east, dry forests in the west, and swamp forests. These species are sometimes found in relatively dense stands at elevations ranging from 500 to 1,500 m above sea level. However, species of this genus are absent from the semi-arid regions of southern Madagascar. According to the Razafimandimbison's taxonomic studies (2002), the genus *Breonia* comprises twenty species that occur exclusively in Madagascar, eight of which were newly described in that study [10]. Despite its endemic occurrence, the genus *Breonia* remains poorly investigated from both phytochemical and biological perspectives. To the best of our knowledge, no studies addressing the chemical composition or biological activities of *Breonia* species belonging to this genus have been reported in the scientific literature to date.

To addres this knowledge gap, the present study focused on *Breonia perrieri* Homolle, an endemic Malagasy species with several local names, including "Hazombalotra," "Marotsaka," "Molompangady," "Valotra," "Vavalotra," "Valotsy," "Valitsy," "Volatra," and "Voakiringy," and commonly referred to as "Breonie" in French. This species is native to the northwestern dry deciduous forests of Madagascar [11]. It is a medium-sized, deciduous tree that reaches approximately 8–15 m in height, with a straight, cylindrical trunk that is up to 70 cm in diameter. Its fruit consists of multiple berries fused into a syncarpous infructescence that is approximately 2 cm in diameter; each berry contains up to four seeds. The fruits are large, fleshy, aggregated, indehiscent, and syncarpous. The ellipsoid seeds are, distinctly flattened, and have provided with rudimentary wings at their base. Flowering occurs annually between November and January, followed by fruiting in January. Fruit maturation takes place from January to March [10,12]. Several endemic Malagasy animals consome the fruits including the brown lemur (*Eulemur fulvus rufus*), the crowned sifaka (*Propithecus coronatus*), and the flat-shelled tortoise (*Pyxis planicauda*). These species occur in western and eastern Madagascar and themselves considered threatened or near-threatened [13].

According to ethnobotanical surveys and available literature, the wood of *B. perrieri* is widely used in local carpentry and construction, as well as in the production of furnitures, bridges, boats, and handicrafts. This heavy timber has a pale yellow to brownish-beige or pinkish-beige heartwood, and a density ranging from 800 to 940 kg/m^3 at 12 % moisture content. It is characterized by its high hardness and difficulty in nailling [12]. It is also suitable for flooring, carved objects, woodturning, wheel manufacturing, and railway sleepers. Furthermore, the wood exhibits moderate resistance to termites and good resistance to fungal attacks [14].

Despite its threatened conservation status, no traditional medicinal uses or scientific data concerning the biological properties of this species have been reported to date. Consequently, conserving and regenerating *B. perrieri* are important challenges because this species is a valuable component of Madagascar's biodiversity and a potential resource for the country's sustainable economic development. Therefore, the present study aimed to promote the valorization of *B. perrieri* through comprehensive phytochemical and pharmacological investigations, as well as by disseminating the first scientific data on this species. This will contribute to its conservation and sustainable management.

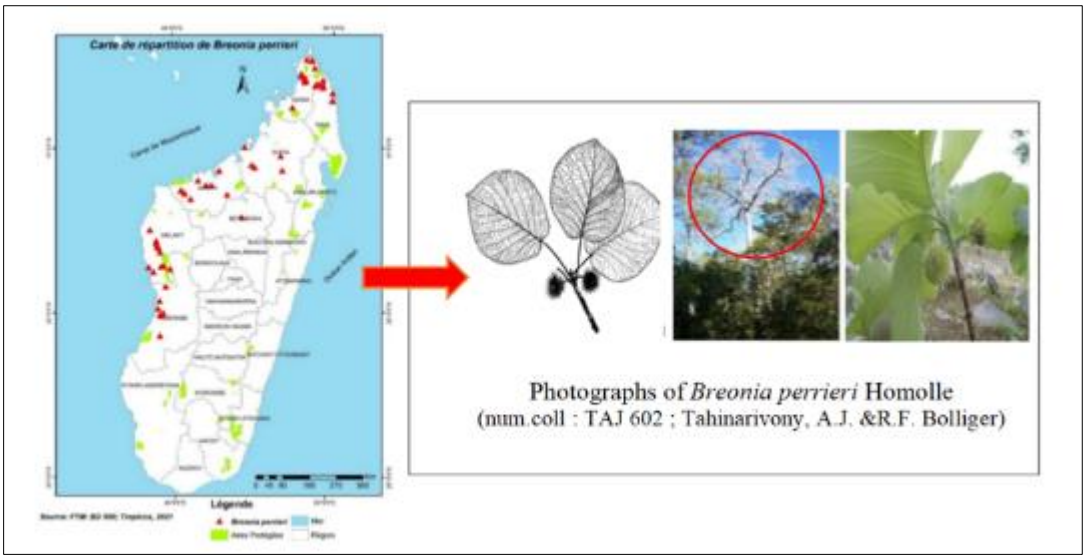


Figure 1 Distribution Map and photographs of *Breonia perrieri* Homolle in Madagascar (Rakotoarisoa F.M)

2. Material and methods

2.1. Plant collection

In April 2018, plant samples consisting of bark, wood, and leaves were collected in Analamanitra, Mitsinjo Municipality and District, Boeny Region, Mahajanga Province, Madagascar, by Rakotoarisoa Falitiana Marrino, a botanist at the CNARP (National Center for Pharmaceutical Research Applications). A voucher specimen was deposited in the Herbarium of the Department of Botany under the reference number RFM35. Table 1 provides detailed informations about the plant material.

Table 1 Plant material information

Scientific name	Vernacular name	Plant family	Plant organ and code	Geographic coordinates
Breonia perrieri (RFM35)	Lovato (Dialecte Sakalava)	RUBIACEAE	Bark RFM35-BK1	Analamanitra,Mitsinjo Municipality and District, Boeny Region, Madagascar -Latitude: 16° 03' 03.7''S -Longitude: 045° 48' 33.0''E -Altitude: 13 m (GPS)
			Leaves RFM35-LF1	
			Wood RFM35-WD1	

Department of Botany (CNARP)

2.2. Phytochemical Analysis Methods

2.2.1. Preliminary Processing

After collection, the different plant organs were air-dried and then ground into a fine powder using a mechanical propeller grinder. The powdered samples were stored in sealed bags until they were used in the various analyses.

2.2.2. Preparation of Crude Extracts

Extraction was carried out by macerating the plant powder in methanol at room temperature. Forty grams (40 g) of each dried powder sample were immersed in the solvent for 48 hours. The resulting macerates were filtered, and the residual plant material (marc) was re-suspended in fresh solvent and subjected to further extraction. The combined filtrates were concentrated under reduced pressure using a rotary evaporator equipped with a water bath maintained

at 40 °C to obtain the crude extracts. The process was repeated until a constant extract weight was achieved [15]. The extraction yield was calculated using the following equation:

$$Yield\ of\ extract\ (\%) = \frac{weight\ of\ extract}{weight\ of\ plant\ material}\ x\ 100$$

2.3. Thin Layer Chromatography (TLC)

TLC is a chromatographic technique that uses a thin stationary phase supported by an inert backing to separate the components of a mixture into well-defined, and well-separated spots. Like all chromatography methods, this technique is based on the principle that a compound will have different affinities for the mobile and stationary phases, affecting the speed at which it migrates [16,17].

The following procedure was used to perform thin-layer chromatography (TCL) on the crude extract of *B. perrieri* (bark, wood, and leaves). First, a quantity of 10 mg of the extract was dissolved in 1 ml of methanol to creat a diluted extract solution. Then, 10 µl of it was deposited onto an Aluminum silica gel plate (Merck 60 F254), which was dried prior to being introduced into the migration tank. The eluent system, consisting of CH_2Cl_2/MeOH (85:15), AcOEt/MeOH (90:10) and AcOEt/MeOH (85:15) served as the migration solvent. The obtained TLC chromatograms were then observed under 254- and 365-nm wavelengths using an ultraviolet lamp. After spraying the plate with 10 % of vanilline perchlorique sulfurique, the retention factor (Rf) values of all spots were determined.

2.4. Phytochemical screening

Phytochemical screening is an essential step in identifying the major classes of chemical constituents present in the plant material [18]. This test is based on the formation of either insoluble (precipitation) complexes or colored complexes. These test are performed directly on plant powders or crude extracts. Methanolic crude extracts and plant powders were used for phytochemical screening [19,20,21].

2.5. Biological Analysis Methods

2.5.1. Evaluation of Antibacterial Activity

The antimicrobial properties of crude extracts from different parts of *B. perrieri* (bark, leaves, wood) were evaluated against several pathogenic reference strains available at the Microbiology Laboratory of the National Center for Pharmaceutical Research Applications (CNARP) of Madagascar. The experiment was conducted out in two stages, following the methods developed by Ngameni *et al*. (2009) and Rakotoarisoa *et al.* (2021), with some modifications [22,23].

The first step involves a semi-quantitative disc diffusion method on solid medium. This is based on the presence or absence of a bacterial growth inhibition zone diameter (IZD) around a filter paper disc impregnated with the test product at a concentration of 1 mg/disc. In the second step, plant extracts showing positive results (IZD > 8 mm) were tested using the broth microdilution method, performed in 96-well microplates, to determine the Minimum Inhibitory Concentration (MIC), the Minimum Bactericidal Concentration (MBC), and the Minimum Fungicidal Concentration (MFC), according to Kuete *et al.* (2009) [24,25]. All tests were performed in triplicate to ensure reproducibility.

The microorganisms-test consisted of six Gram-positive bacteria (*Staphylococcus aureus* ATCC 11632, *Bacillus cereus* LMG 6910, *Listeria monocytogenes* ATCC 19114, *Streptococcus pyogenes* ATCC 19615, *Streptococcus pneumoniae* ATCC 6305, *Clostridium perfringens* ATCC 13124), six Gram-negative bacteria (*Salmonella enterica* subsp. ATCC 1376, *Shigella flexneri* ATCC 12022, *Proteus mirabilis* ATCC 35659, *Yersinia enterocolitica* ATCC 23715, *Enterobacter cloacae* ATCC 13047, *Enterobacter aerogenes* ATCC 13048), and one yeast species (*Candida albicans* ATCC 10231). The reference antimicrobial agents, used as positive controls, to evaluate and compare the antimicrobial activity of the plant extracts against the tested microorganisms were: doxycycline (30 µg/disc), streptomycin (10 µg/disc), neomycin (30 µg/disc), and miconazole (50 µg/disc). The antimicrobial result was interpreted as folllow:

- According to the standard of Ponce *et al*. (2003), the sensitivity of the strains to the extracts was determined as follows : bacteria were considered not sensitive (-) for an inhibition zone diameter IZD ≤ 8 mm, sensitive (+) for 9 to 14 mm, very sensitive (++) for 15 to 19 mm, and extremely sensitive (+++) for IZD > 20 mm [26].
- According to Dalmarco *et al*. (2010) , the activity of a crude extract can be classified, based on its MIC value (µg/ml), as “Excellent (MIC < 100)”, “Moderate (100-500)”, “Weak (500-1000)” or “Inactive (>1000)”. Based on this measurement, the nature of the observed effect can be classified : an extract is considered bactericidal

or fungicidal when the MBC (or MFC) /MIC ratio is less than or equal to 4, and bacteriostatic or fungistatic when the ratio is greater than 4 [27].

2.6. Evaluation of Free Radical Scavenging Activity

The free radical scavenging activity of the methanolic extracts was determined using the DPPH (2, 2 Diphenyl-1Picrylhydrazyl) method described by Awika *et al*. (2003), Popovici *et al*. (2009), and Rakotoarisoa *et al*. (2025), with some modifications [28,29,30]. This experiment is based on radical degradation, whereby the addition of an antioxidant reduces the radical and causes the mixture to change color. This evaluation involved two complementary stages, using qualitative and quantitative methods.

2.6.1. Qualitative Evaluation

To accomplish this step, 30 µl of each sample, at a concentration of 1 mg/10 µl, was applied to silica gel 60 F254 glass TLC plates (normal-phase chromatography). The plates were developed using an appropriate solvent system, them sprayed with a 25 % methanolic DPPH solution [31]. The results were visually recorded after 30 min of incubation.

2.6.2. Quantitative Evaluation

The free radical scavenging activity of the extracts identified as active during the TLC-bioautography screening (+++) was evaluated quantitatively using a spectrophotometric DPPH assay, following the procedures described by Popovici *et al*. (2009) and Rakotoarisoa *et al*. (2026). [23,29]. This step involved measuring the absorbance (Abs), or optical density, of a given chemical substance in solution [32]. The intensity of the color produced is proportional to the antioxidants's ability to reduce the DPPH radical in the medium. The degree to which the DPPH radical is discolored depends on the concentration of the different extracts tested. The rate of color loss is directly proportional to the antioxidant activity of the hydrogen-donating compounds. Each sample was analyzed in triplicate, and the mean absorbance value was calculated.

Accordingly, a 4.5 % methanolic DPPH solution was prepared and stored at −20 °C in the dark until use. In dry test tubes, 200 µl of each extract concentration (ranging from 62.5 to 1000 µg/ml) were mixed with 3800 µl of the DPPH solution (4.5 %). For each concentration, a blank consisting of 3800 µl of DPPH solution and 200 µl of methanol was prepared. After incubation for 30 min in the dark at room temperature, the absorbance (Abs) was measured at a wavelength of 517 nm using a spectrophotometer. The absorbance values were then converted into percentage inhibition (PI %), which reflects the DPPH free radical scavenging capacity of the extracts. The antiradical activity of the extracts, corresponding to their ability to scavenge the DPPH free radical, was calculated using the following equation [33]:

$$PI\ \% = [(Abs\ blank - Abs\ sample)/Abs\ blank] \times 100$$

Where, PI %: Percentage inhibition; Abs blank : absorbance of the control (DPPH solution without extract); Abs sample : absorbance of the test sample containing the extract

The IC_{50} value, which is defined as the concentration required to inhibit 50 % of DPPH radicals, was determined from the inhibition curve and is used as an indicator of antioxidant activity [34,35]. The percentage inhibition (PI %) values corresponding to the different concentrations of the extracts or the reference antioxidant (α-tocopherol) were plotted to graphically determine the IC_{50} value [36,37,38]. This value was calculated using a regression equation established wiht these parameters. Generally, the lower the IC_{50} value, the greater the reducing power and antioxidant potential of the extract [39].

2.7. Statistical Analysis

The statistical analysis of the raw data was performed using Principal Component Analysis (PCA). Results are expressed as mean values ± standard deviations from three independent determinations. Thus, differences were considered statistically significant when $p < 0.05$.

3. Results and discussion

3.1. Chemical Results

3.1.1. Extraction Yield

The crude extraction yields obtained by methanolic maceration from the different plant organs (bark, leaves, wood) are summarized in Table 2.

Table 2 Results of methanolic extraction of de *B. perrieri* organs

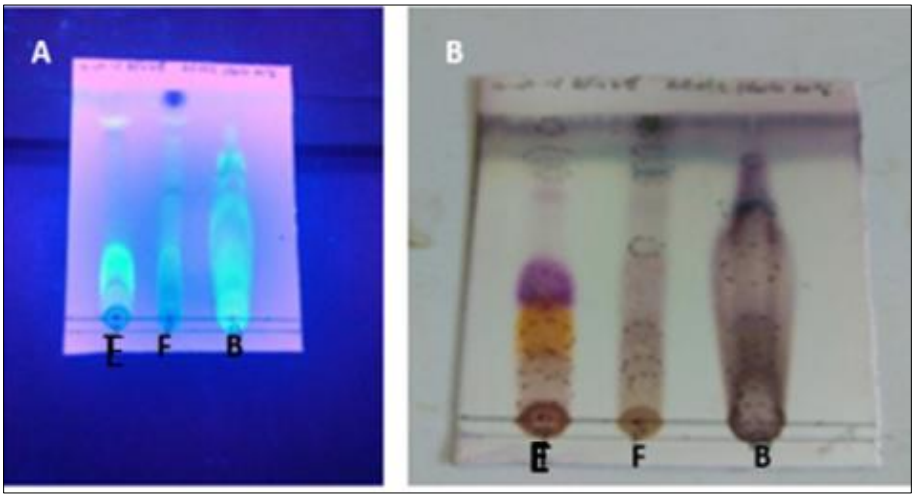
Plant code	Mass of plant powder (g)	Mass of obtained extract (g)	Extraction yield (%)	Extract color
Bark (RFM 35-BK1)	40	8.25	20.63	Orange red powder
Leaves (RFM 35-LF1)	40	2.72	6.79	Green powder
Wood (RFM 35-WD1)	40	3.55	8.87	Brown powder

According to these results, the extraction yields of crude extract varied considerably depending on the plant organ used.

- The bark extract (RFM35-BK1) had the highest extraction yield at **20.63 %**. This high value suggests an especially high concentration of methanol-soluble compounds. The extract's orange-red color may be associated with the presence of pigmented phenolic compounds and anthraquinones [40].
- In contrast, the leaf extract (RFM35-LF1) had the lowest yield (**6.79 %**) and was obtained as a green powder, likely due to chlorophylls and other plant pigments [41].
- The wood extract (RFM35-WD1) had an intermediate yield of **8.87 %**, producing **3.55g** of brown powder. This moderate rate can be explained by the high proportion of lignified and structural compounds in wood tissues, which are generally less extractable [42].

Overall, these results suggest that bark is the plant organ with the highest concentration of compounds that can be extracted by cold methanolic maceration. The observed differences in extraction yields among the various organs may be attributed to the unequal distribution of secondary metabolites and differences in tissue structure and biochemical composition.

3.1.2. Chromatographic Profiles of the Extracts



A : observation under UV light ; B : TLC plate after revelation ; (Mobiles phases : CH_2Cl_2 / MeOH (85 : 15), AcOEt/ MeOH (90 :10), and AcOEt/ MeOH (85 :15)

Figure 2 TLC profils of *B. perrieri* extracts: E (bark), F (leaves), B (wood)

The Thin-Layer Chromatography analysis of the bark (E), leaf (F), and wood (B) extracts of *B. perrieri* visualized under UV light (A) and after revelation with a chemical reagent (B) is show in the following figure 2. The number of compounds in the plant extract is revealed by the positions and characteristics of the spots.

- Under UV illumination (Figure 2A), several fluorescent bands with different migration distances (Rf values) were observed, indicating the presence of multiple phytochemical constituents in the three extracts. Compared to the leaf extract, the bark and wood extracts exhibited more intense and numerous fluorescent spots, suggesting a greater diversity or concentration of UV-active compounds, such as coumarins, flavonoids, anthraquinones, and other phenolic derivatives.
- After spraying with the revelation reagent (Figure 2B), colored bands ranging from yellow and orange to purple and dark brown appeared on the chromatograms. These color variations indicate the presence of chemically distinct groups of secondary metabolites. The bark extract showed several strongly colored spots, confirming its richness in polyphenolic and anthraquinone-type compounds. The leaf extract revealed fewer but distinct spots, that may correspond to flavonoids, anthocyanins, and alkaloids. The wood extract presented a broad and intense dark zone, suggesting the presence of highly concentrated phenolic compounds or tannins.

The differences in band intensity and migration patterns among the three extracts demonstrate variations in their phytochemical composition. Similar bands observed at comparable Rf values may indicate the presence of metabolites common to certain plant organs, while unique bands reflect organ-specific compounds. Overall, the results of the TLC analysis show that the bark, leaves, and wood of *B. perrieri* possess distinct chemical fingerprints characterized by diverse secondary metabolites.

3.1.3. Qualitative Phytochemical Screening of the crude extracts

Table 3 summarizes the phytochemical screening results of the methanolic extracts (RFM35-BK1, RFM35-LF1, RFM 35-WD1) from bark, leaves, and wood of *B. perrieri*.

Table 3 Phytochemical screening results performed on the bark, leaves, and wood of *B. perrieri* extracts

Chemical families	**Characterization reagents**	**RFM35-BK1**	**RFM35-LF1**	**RFM 35-WD1**
Coumarins	NaOH, UV lamp	+++	-	+++
Anthraquinones	NH_4OH	+++	-	-
Anthracene glycosides	HCl ou H_2SO_4, $FeCl_3$, NH_4OH	+	+	-
Flavans	Chlorhydric Vanillin	-	-	-
Anthocyanins	Cold HCl	++	+++	-
Leucoanthocyanes	Hot HCl	+	-	-
Flavones	HCl, Mg, Isoamyl Alcohol	-	-	+
Flavonols		-	-	-
Flavanones Flavanonols	HCl, Mg	-	+++	+++
Polyphenols	Gelatin	+++	+++	-
Tannins	NaCl, Gelatin	-	++	++
Hydrolyzables tannins	$FeCl_3$	+++	+++	+++
Condensed tannins		-	-	-
Cyanogenic glycosides	$CHCl_3$; Sodium picrate	-	-	-
Alkaloids	KI, I_2, $HgCl_2$, Bi $(NO_3)_3$, Tartaric acid	+	+++	-
Iridoids	HCl, $CuSO_4$, Glycerol	-	-	-
Triterpenoids	Acetic anhydride, H_2SO_4, Antimony	-	+++	-
Steroids		++	-	-
Unsaturated sterols	H_2SO_4	-	-	-

Lactonic steroids	Picric acid, KOH or NaOH, 3,5 Dinitrobenzoic acid	++	+++	+++
Cardenolide	H_3PO_4, CCl_3COOH	-	-	-
2-Deoxysugar heterosides	H_3PO_4, CCl_3COOH	+	++	+
Saponins	Foam height	+++	+	+++
Antracenosides	NH_4OH	+++	-	+++
Polysaccharides	90° Ethanol	+++	-	-

- : : no reaction observed (negative test) ; + : weak coloration or slight precipitate formation ; ++ : distinct coloration or abundant precipitate formation ; +++ : intense coloration, immediate flocculation, or foam height greater than 5 cm.

Qualitative phytochemical screening of extracts from the three organs of *Breonia perrieri* revealed a high diversity of secondary metabolites, with a variable distribution among examined plant organs. This variability suggests metabolic specialization of the different plant tissues.

- The bark extract (RFM35-BK1) exhibited the most diverse phytochemical profile. It was particularly rich in coumarins, anthraquinones, polyphenols, hydrolyzable tannins, saponins, polysaccharides, and anthracene derivatives. The presence of anthraquinones, coumarins, and phenolic compounds simultaneously is often associated with the antimicrobial, antioxidant, and anti-inflammatory activities reported in several RUBIACEAE species [43]. The detection of steroids and lactonic steroids further suggests an interesting biological potential.
- The leaf extract (RFM35-LF1) was characterized by its high alkaloids, flavanones/flavanonols, anthocyanins, and hydrolyzable tannins content. Alkaloids are a class of compounds well known for their wide range of pharmacological activities, including antibacterial, antiparasitic, and cytotoxic effects [44]. Despite its low extraction yield, the leaf extract exhibited considerable qualitative richness in chemical constituents. This indicates that the amount of extract obtained is not necessarily correlated with the chemical diversity of secondary metabolites.
- The wood extract (RFM35-WD1) had a less complex phytochemical profile, but was characterized by a high abundance of coumarins, hydrolyzable tannins, lactonic steroids, saponins, and anthracene derivatives. The presence of flavones and flavanones/flavanonols also suggests a potential antioxidant activity [45]. Though the wood extract is less rich in secondary metabolites than the bark or leaf extracts, it still contains several classes of biologically active compounds.

These phytochemical screening results were further confirmed by Principal Component Analysis (PCA) (Figure 3). PCA revealed clear chemical differentiation among the three plant organs. The bark extract was characterized by an abundance of phenolic and anthracene-derived compounds, while the leaf extract was associated primarily with alkaloids and triterpenoids. The wood extract exhibited an intermediate phytochemical profile, influenced primarily by flavanones/flavanonols, and saponins. This clear separation suggests that the three samples have different phytochemical compositions, which could explain variations in their biological activities.

Several metabolites were absent from all the plant organs investigated, including flavans, flavonols, condensed tannins, cyanogenic glycosides, iridoids, cardenolides, and unsaturated sterols. The absence of cyanogenic glycosides is particularly noteworthy from a toxicological perspective, as it suggests a low risk associated with cyanide release [46].

On the whole, these findings demonstrate that *Breonia perrieri* constitutes an important source of bioactive secondary metabolites. The predominance of polyphenols, hydrolyzable tannins, flavonoids, alkaloids, coumarins, and saponins may explain the antibacterial and antioxidant activities observed during the biological assays. Moreover, these results are consistent with the studies of Taskova *et al*. (1997) and Rasoarivelo *et al*. (2017) regarding the taxonomic markers characteristic of the RUBIACEAE family [47]. These compounds may act individually or synergistically to produce the pharmacological effects demonstrated in the present study. Such chemical richness confers significant potential for the valorization of this species in pharmacognosy and the future development of phytomedicines, while further emphasizing the importance of its conservation due to its endemic and threatened status in Madagascar.

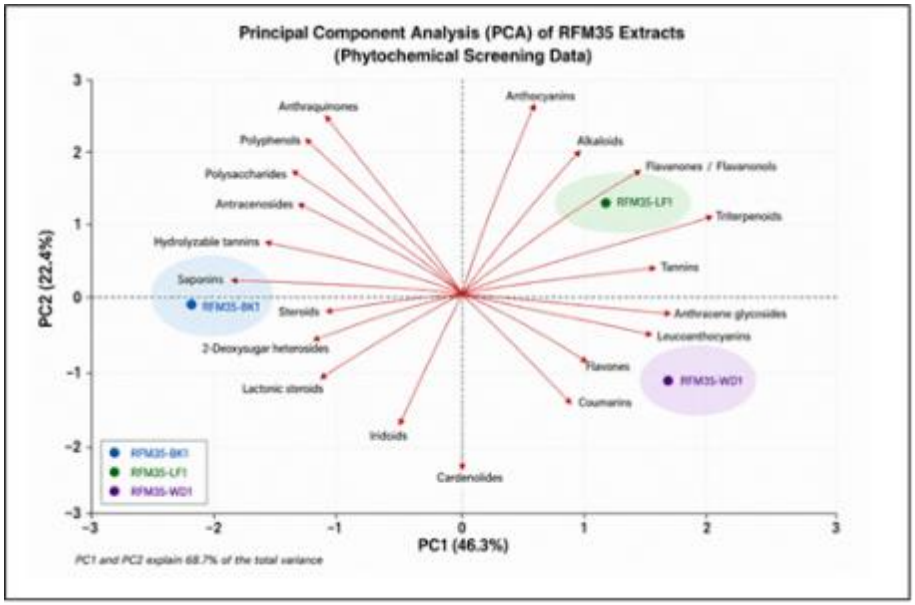


Figure 3 Representative of phytochemical variability among B. perrier parts extracts using Principal Component Analysis

3.2. Biological Results

3.2.1. Antibacterial Activity

Antibiogram Results

The table 4 shows the antimicrobial activity of the crude extracts of *B. perrieri* bark, leaves, and wood, expressed as inhibition zone diameter (IZD, mm ± standard deviation).

Table 4 Inhibition zone diameter of methanolic extracts from tree parts of *B. perrieri* against pathogenic microorganisms

Microorganisms	**Value of the Inhibition Zone Diameter (IZD mm ± standard deviation)**					
	RFM35-BK1	**RFM35-LF1**	**RFM 35-WD1**	**T1**	**T2**	**T3**
Salmonella enterica	6 ± 0	6 ± 0	6 ± 0	35	NT	NT
Shigella flexnerii	6 ± 0	7 ± 0	6 ± 0	27	NT	NT
Proteus mirabilis	6 ± 0	6 ± 0	6 ± 0	32	NT	NT
Yersinia enterocolitica	6 ± 0	7 ± 0	6 ± 0	34	NT	NT
Enterobacter cloacae	6 ± 0	6 ± 0	6 ± 0	10	NT	NT
Enterobacter aerogenes	6 ± 0	6 ± 0	6 ± 0	16	NT	NT
Staphylococcus aureus	6 ± 0	6 ± 0	6 ± 0	23	NT	NT
Bacillus cereus	6 ± 0	7 ± 0	6 ± 0	33	NT	NT
Listeria monocytogenes	6 ± 0	7 ± 0	6 ± 0	27	NT	NT
Clostridium perfringens	6 ± 0	6 ± 0	6 ± 0	26	NT	NT
Streptococcus pyogenes	6 ± 0	6 ± 0	6 ± 0	NT	27 ± 0.3	NT
Streptococcus pneumoniae	**11 ± 0**	**11 ± 0**	**11 ± 0**	NT	26 ± 0	NT
Candida albicans	6 ± 0	6 ± 0	6 ± 0	NT	NT	23 ± 0.6

RFM35-BK1 : bark ; RFM35-LF1 : leaves; RFM 35-WD1 : wood ; T1 : Doxycycline (30 µg/disc) ; T2 : Streptomycin (10 µg/disc) ; T3 : Miconazole (50 µg/disc); NT: Non-Tested

In general, the three plant extracts (bark, leaves, wood) exhibited very weak or no antimicrobial activity against the tested microorganisms. Indeed, inhibition zone diameters of 6 ± 0 mm indicating an absence of an inhibitory effect. Slightly larger inhibition zones (7 ± 0 mm) were observed for the leaf extract (RFM35-LF1) against *Shigella flexnerii*, *Yersinia enterocolitica*, *Bacillus cereus*, and *Listeria monocytogenes*, which suggest a very low antibacterial activity. Of all the tested microorganisms, *Streptococcus pneumoniae* was the most sensitive strain to the plant extracts, with inhibition zones of 11 ± 0 mm were observed for all three extracts. This result indicates a moderate susceptibility of this Gram-positive bacterium to the phytochemical constituents present in the extracts. The positive controls demonstrated strong antimicrobial activity with large inhibition zones ranging from 10 to 35 mm against bacterial strains, confirming the validity of the experimental conditions. The weak antimicrobial activity of the crude extracts may be attributed to several factors, including low concentrations of active compounds, poor diffusion of certain phytochemicals through the agar medium, and antagonistic interactions between metabolites in crude mixtures. Nevertheless, the moderate activity observed against *Streptococcus pneumonia* may be explained by the presence of flavonoids, tannins, coumarins, anthraquinones, alkaloids, and saponins identified during phytochemical screening.

In conclusion, the crude extracts of *B. perrieri* exhibited minimal antimicrobial activity under the tested conditions. Determining the minimum inhibitory concentrations (MICs) was intended to more accurately evaluate this species' antimicrobial potential.

3.2.2. Micro-well Dilution Results

The following table 5 presents the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the crude extracts of *B. perrieri* bark, leaves, and wood against the sensitive strain *Streptococcus pneumoniae*.

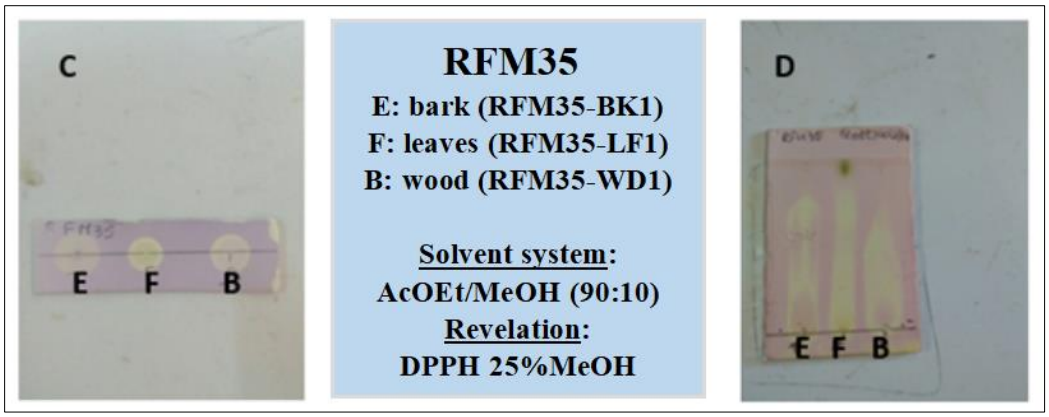
Table 5 MIC and MBC values of the sensitive strain against the crud extracts of *B. perrieri*

Extract	**Sensitive strain: *Streptococcus pneumoniae***			
	MIC (µg/ml)	MCB (µg/ml)	MIC/MCB ratios	Class of activity
RFM35-BK1	250	>1,000	>4	Moderate, Bacteriostatic
RFM35-LF1	250	>1,000	>4	Moderate, Bacteriostatic
RFM35-WD1	250	>1,000	>4	Moderate, Bacteriostatic

The three parts of *B. perrieri* exhibited identical MIC values of 250 µg/ml, indicating that they all possess a comparable inhibitory effect against the growth of *S. pneumoniae*. According to the commonly accepted antimicrobial activity criteria of Dalmarco *et al*. (2010) for plant extracts, MIC values between 100 and 500 µg/ml are generally considered to reflect moderate antimicrobial activity [27]. Therefore, the three extracts demonstrated moderate antibacterial potency against this strain. However, the MBC values for all extracts were gearter than 1,000 µg/ml. Since the MBC/MIC ratio exceeded 4, the extracts were classified as bacteriostatic rather than bactericidal. These results suggest that the extracts were unable to completely kill the bacterial cells at the tested concentrations. The high MBC values indicate that much larger concentrations would be required to achieve a bactericidal effect. The similarity observed among the extracts of the three plant parts may indicate that the antibacterial compounds active against *S. pneumoniae* are distributed throughout the different organs of *B. perrieri*. The bacteriostatic effect may be related to the presence of bioactive secondary metabolites such as flavonoids, tannins, coumarins, alkaloids, anthraquinones, and saponins, which were identified during phytochemical screening and are known for their antimicrobial properties. Overall, these results confirm the moderate antibacterial potential of *B. perrieri* crude extracts against *Streptococcus pneumoniae*. However, the absence of bactericidal activity suggests that purifying and concentrating the active constituents may be necessary to enhance their antimicrobial effectiveness.

3.2.3. Antioxidant and Free Radical Scavenging Activity

Figure 4 presents the results of the free radical scavenging potential of the crude extracts from thin-layer chromatography (TLC) bioautography of the bark (E), leaves (F) and wood (B). The chromatographic separation was carried out using the solvent system AcOEt/MeOH (90:10), followed by revelation with 25 % DPPH in methanol.



C : unmigrated spot ; D : developed spot

Figure 4 Profile chromatography of free radical scavenging potential of *B. perrieri* crude extracts

The appearance of yellow or pale spots against the purple background after DPPH spraying indicates the presence of compounds with free radical scavenging activity. These compounds can reduce the stable DPPH radical, resulting in discoloration zones corresponding to antioxidant-active metabolites.

- In Figure 4D, show that the bark (E) and wood (B) extracts had larger and more intense discoloration zones than the leaf extract (F). This suggests that these organs have a higher antioxidant potential. This antioxidant activity may be due to phenolic compounds, such as polyphenols, tannins, flavonoids, coumarins, and anthraquinones, which were previously identified during phytochemical screening.
- The bark extract showed several distinct active bands, indicating the presence of multiple antioxidant constituents with different polarities and migration behaviors. The wood extract also displayed notable antioxidant bands, confirming the contribution of its phenolic metabolites to radical scavenging activity. In contrast, the leaf extract revealed weaker or fewer active spots, suggesting a comparatively lower concentration of antioxidant compounds under the tested conditions.

Differences in spot intensity and migration patterns among the three extracts demonstrate variability in the qualitative and quantitative compositions of antioxidant metabolites in different plant organs. Similar migration levels between certain spots may indicate the presence of shared antioxidant compounds among the extracts. Overall, TLC-DPPH bioautography confirms the antioxidant potential of *B. perrieri* extracts, particularly in the bark and wood. This result supports the phytochemical findings that these plant parts are rich in phenolic secondary metabolites. The following table 6 presents the quantification of their free radical scavenging capacity, determined by the IC_{50} value, for comparison with that of α-tocopherol.

Table 6 Quantification of the free radical scavenging activities of the crude extracts from the leaves, bark and wood of *B.perrieri* against DPPH

Plant extract	TLC- bioautography results	IC_{50} value (µg/ml)
RFM35-BK1	+++	6.56 ± 0.2
RFM35-LF1	+++	17.46 ± 0.1
RFM 35-WD1	+++	99.74 ± 0.44
α-tocopherol	++++	11.43 ± 0.1

α-Tocopherol used as the positive control; NT = Not tested; IC_{50} = concentration required to inhibit 50 % of free radicals; +++: high intensity

Table above presents the antioxidant activity of the crude extracts as IC_{50} values,with α-Tocopherol was used as the reference antioxidant standard.

The TLC-bioautography results revealed that all of the plant extracts exhibited strong antioxidant activity (+++), indicating the presence of compounds capable of scavenging free radicals. Subsequently, a quantitative evaluation based on IC_{50} values confirmed significant differences among the extracts. The bark extract (RFM35-BK1) exhibited the strongest antioxidant activity with an IC_{50} value of 6.56 ± 0.2 µg/ml. This value is lower than that of α-tocopherol (11.43

± 0.1 µg/ml), suggesting that the bark extract has a greater radical scavenging capacity than the reference antioxidant under these experimental conditions.

- The leaf extract (RFM35-LF1) exhibited significant antioxidant activity, with an IC_{50} value of 17.46 ± 0.1 µg/ml. Although it is less active than the bark extract and α-tocopherol, it still demonstrates a strong capacity to neutralize free radicals.
- In contrast, the wood extract (RFM35-WD1) displayed the weakest antioxidant activity, with an IC_{50} value of 99.74 ± 0.44 µg/ml. Since antioxidant potency is inversely proportional to IC_{50} values, this result indicates a considerably lower free radical scavenging capacity compared than the bark and leaf extracts.
- The superior antioxidant activity of the bark extract may be attributed to its high content of polyphenols, hydrolysable tannins, anthraquinones, coumarins, and saponins, as revealed by the phytochemical screening [48,49]. Phenolic compounds are well known for their ability to donate hydrogen atoms or electrons, thereby stabilizing reactive oxygen species and preventing oxidative damage. Several studies have reported that the antioxidant potential of plant extracts is closely associated with the presence and abundance of phenolic constituents [50,51,52].

These findings demonstrate that the bark of *B. perrieri* is the richest source of antioxidant compounds among the studied organs. It may be represent a promising natural source of bioactive molecules for use in the pharmaceutical, nutraceutical, and food industries.

4. Conclusion

The findings of this study significantly contribute to the valorization of *Breonia perrieri* Homolle. To the best of our knowledge, these data are the first to describe the phytochemical composition and biological activities of this species. Overall, the results suggest that *B. perrieri* is a promising source of bioactive compounds for developing novel anti-infective and antioxidant therapeutic agents. Beyond its economic value as a timber species, *B. perrieri* may be an important natural resource for preventing or managing disorders associated with oxidative stress and infections caused by *Streptococcus pneumoniae*, including pneumonia, otitis, and meningitis. Nevertheless, the biological activities reported in this study were evaluated exclusively on crude extracts, and the plant organs were not collected at different phenological stages. This is important because the biosynthesis and accumulation of secondary metabolites may vary throughout plant development. Further investigations are therefore required to isolate and identify the compounds responsible for the observed activities, as well as elucidate their mechanisms of action, bioavailability, safety profile, and *in vivo* efficacy. Bioassay-guided isolation of active constituents, particularly from the bark extract, which exhibited the most promising biological activities, is an essential step toward understanding the pharmacological potential of *B. perrieri* and its future use in pharmaceuticals.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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