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Organ-Specific Chemical Diversity and Biological Activities of Methanolic Extracts from *Gaertnera phanerophlebia* Baker (RUBIACEAE)

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Abstract

This study focused on the comparison of the chemical and biological profiles of the methanolic extracts from the stems, leaves, and leafy twigs of *G. phanerophlebia* Baker. This approche is fundamental for the valorization of natural resources. The extraction yields revealed more extractable compounds than the other parts, with the highest yield of 15.37 % found in the leaves, followed by the leafy twigs (11.62 %) and the stems (5.25 %). Despite their lower yield, the leafy twigs exhibited the most significant biological activities. The crude extract from the leafy twigs (ST 380 BTRF) manifested high antiplasmodial activity against *Plasmodium falciparum* with an IC_{50} of 16.63 µg/ml compared to the stem and leaf extracts. This extract also demonstrated remarkable antibacterial activity, particularly against Gram-positive bacteria such as *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*, as well as the best antimicrobial effects with bactericidal activity against certain strains. However, no antifungal activity was observed against *Candida albicans* in any part of the plant. The evaluation of antioxidant activity using the DPPH assay revealed strong free radical scavenging capacity for all plant part extracts, especially the leafy twigs, whose IC_{50} (12.56 µg/ml) was close to that of α-tocopherol. These results suggest that *G. phanerophlebia* Baker, particularly the leafy twigs, is a promising source of bioactive compounds with pharmacological potential. Additionally, this study also confirmed that secondary metabolites are not uniformly distributed, and each plant organ possesses its own chemical profile and specific biological activities.

Keywords: *Gaertnera Phanerophlebia*; Methanolic Extracts; Stems; Leaves; Leafy Twigs

1. Introduction

Madagascar is renowned worldwide for its richness in medicinal plants [1]. For the majority of its population, these plants are still one of the most practical means of treating health problems [2]. However, depending on the traditional knowledge and practices of different regions, medicinal plants are most often used alone or in complex mixtures, involving one or more plant parts to prevent or treat various diseases. In traditional medicine, therefore, the same plant may be used to treat different pathologies depending on the method of preparation and, especially, the part of the plant used (e.g., stems, leaves, roots, bark) [3]. This is the case with the Madagascar periwinkle, *Catharanthus roseus*, whose roots are used to treat diabetes, and whose leaves are used for liver disorders and malaria [4]. For several decades, phytochemical and pharmacology research has focused on secondary plant metabolites, such as alkaloids, flavonoids,

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terpenes, and phenolic compounds, due to their numerous biological properties. The RUBIACEAE family is recognized for its chemical richness and therapeutic importance. This family includes species that are traditionally used for their antiplasmodial, antimicrobial, and antioxidant properties [5].

In a context marked by the emergence of resistance to conventional treatments, the search for new active substances derived from natural resources has become a scientific priority. Bacterial infections and malaria caused by *Plasmodium falciparum*, as well as oxidative stress, remain major public health concerns, especially in tropical regions [6]. Therefore, the study of medicinal plants appears to be a promising approach for identifying new compounds with therapeutic potential.

The study of *G. phanerophlebia* Baker is conducted within this framework. This medicinal species, which belongs to the RUBIACEAE family, is used in the eastern Madagascar as a febrifuge and to promote wounds healing [7]. Though it has received little attention, it could be an interesting source of biologically active secondary metabolites. Furthermore, traditional pharmacopoeia does not specify which part of the plant is active, only recommending its aerial parts. Since bioactive compounds are not uniformly distributed among the different plant organs, the stems, leaves, and leafy twigs of this plant may exhibit distinct chemical profiles and, consequently, different biological activities. Therefore, this comparative study could scientifically, identify the organs richest in bioactive compounds and the most promising for future pharmacological application by quantifying the biological activities and determining the chemical compounds present in each plant part.

2. Materials and Methods

2.1. Plant collection

The fresh parts of *Gaertnera phanerophlebia* Baker (stems, leaves, and leafy twigs) were collected in December 2019 from the Alaotra Mangoro region of Madagascar, specifically from the commune of Ambohibary and the Fokontany of Ampitambe Ambatomainty (Fig.1). Botanists at the National Center for Pharmaceutical Research Applications (CNARP) identified and authenticated the plant. A voucher specimen was archived in the Department of Botany herbarium under the registration number ST380. The three parts of the plant were air-dried then after and subsequently milled into a fine powder using a mechanical grinder.

2.2. Extraction

The extraction-to-exhaustion technique was implemented and the experimental details are provided below. Based on the procedure, 40 g of the powdered plant material underwent methanolic extraction via cold maceration at room temperature using 575 ml of solvent for 24 hours. The mixture was filtered with filter paper, and the filtrate was concentrated in a rotary evaporated with a water bath at 40 °C (Fig.1). The resulting crude extracts was weighted and stored at 4 °C until needed. The extraction yield was calculated using the following formula:

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

Figure 1 Plant collection (1), air-dried plant and extraction method (2,3)

2.3. Phytochemical screening

The characterization techniques vary according to the type of the secondary metabolites being targeted (e.g., alkaloids, coumarins, flavonoids, terpenoids, saponins, polyphenols, polysaccharides, anthraquinones, and anthracenosides). These tests are based on either the formation of insoluble complexes (precipitation reactions) or on the formation of

colored complexes. They are performed directly from plant powders or crude extracts [8]. Methanolic crude extracts and plant powders were used for phytochemical screening.

2.4. Evaluation of antimicrobial activities

The antimicrobial properties of crude extracts from different parts of the plant (stems, leaves, and leafy twigs) 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 (Fig.2), following methods developed by Ngameni *et al*. (2009) and Rakotoarisoa *et al* (2021), with some modifications [9,10].

The first step concerns the semi-quantitative method by disc diffusion on solid medium. It is based on the presence or absence of a bacterial growth inhibition zone around a filter paper disc impregnated with the test product at a concentration of 1 mg/disc. Following an incubation period of 24 hours at 37°C for bacteria and 72 hours at 25°C for yeasts [11], the results are interpreted by measuring the the Inhibition Zone Diameter (IZD in mm). This parameter is proportional to the microorganism's sensitivity: the larger the diameter of the inhibition zone, the more sensitive the strain is [12]. The positive controls consisted of cellulose discs pre-impregnated with the following reference antibiotic or antifungal agents: Doxycycline (30 µg/disc), Miconazole (50 µg/disc), and Neomycin (30 µg/disc). According to the interpretation standard of Ponce *et al*. (2003) [13], the sensitivity of the strains to the extracts was determined as follow: 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.

In the second step, the plant extracts that showed positive results (IZD > 8 mm) were subjected to 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) [14,15]. This approach is essential for quantifying the extract's antimicrobial activity since an antibiogram only provides information on the microorganisms's sensitivity or resistance to the tested product. Therefore, the MIC, MBC and MFC are considered indicators of antimicrobial activity, making it possible to compare the antibiotic's activity against a given microbial strain.

The **MIC** is the lowest concentration of an antibiotic that prevents bacterial growth. This value characterizes the bacteriostatic effect of an antibiotic. 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)" [16].

The **MBC** and **MFC** are defined as the lowest concentration of an antibiotic that destroys 99.9 % of the microbial and fungal population, respectively, after incubation at which no bacterial colonies grow [17]. These values characterize the bactericidal or fungicidal effect of an antibiotic. This is generally determined after 18 hours of contact between the bacterium and the antibiotic. 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. All tests were performed in triplicate to ensure reproducibility [18].

Figure 2 Antimicrobial assay: disc diffusion (1) and microdilution methods (2)

2.5. Evaluation of antioxidant and free radical scavenging activity

The antioxidant 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 [19,20,21]. This test is based on the degradation of the DPPH radical whereby the addition of an antioxidant reduces the radical and causes the mixture to change color (Fig.3).

Figure 3 Antioxydant and free radical scavenging assay: colorimetric method with DPPH (1), spectrophotometer analysis (2)

2.6. Evaluation of anti-plasmodial activity

The assay method was developed by Trager and Jensen (2005) and Ratsimbason *et al*. (2009) [22,23]. The method is based on measuring of the fluorescence of a solution containing the *Plasmodium falciparum* FCM29 parasite after adding a fluorophore (SYBR Green I).

A fluorescent probe, SYBR Green I, which is specific to double-stranded DNA, was used to monitor the intraerythrocytic growth of *Plasmodium falciparum*. The fluorescence of the DNA–SYBR Green I complex is proportional to the amount of DNA present. Therefore, the fluorescence intensity reflects the size of the parasite population. Experiment was realised in ninety-six-well microplates and the fluorescence readings were performed in microplate reader (FLx 800, Biotek) with excitation at 485 nm and emission at 518 nm.

First, the assay involved of maintaining the erythrocytic stage (human red blood cells) of the *Plasmodium falciparum* FCM29 strain in continuous culture [24]. Then, 50 µl of the extract was brought into contact with the parasite at a single concentration of 50 µg/ml for the initial screening. Those extracts that induced more than 75 % inhibition of the parasite population after 72 h of incubation at 37 °C were selected for IC_{50} evaluation using a series of dilutions ranging from 50 µg/ml to 0.33 µg/ml. At the end of each incubation period, blood smears were prepared. Then, the parasitemia was determined for each smear, and the percentage of parasite growth inhibition was calculated using to the formula described by Fidock *et al*. (2004) [25] :

$$\text{Pourcentage of inhibition (\%)} = \left[1 - \frac{\text{Mean parasitemia of the test sample}}{\text{Mean parasitemia of the control}}\right] \text{x}100$$

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. Extraction yield

The extraction results for each plant part are presented in the following table 1.

Table 1 Results of methanolic extraction : stems (a), leaves (b), and leafy twigs (c) of *G. phanerophlebia*

Extract code	Mass of obtained extract (g)	Extraction yield (%)	Extract color
ST380BTT (a)	2.21	5.25	Brown
ST380BTF (b)	6.15	15.37	Green
ST380BTRF (c)	4.65	11.62	Green

These results demonstrate that crude extracts yields varied according to the plant organ used. Leaves (ST380BTF) had the highest yield at 15.37 %, followed by the leafy twigs (ST380BTRF) with an intermediate yield of 11.62 %. Stems (ST380BTT) had the lowest yield at only 5.25 %. This variation suggests that the leaves contain more extractable compounds than stems. In contrast, stems contain more lignified and fibrous tissues, which are have fewer extractable substances, thus explaining their lower yield. The leafy twigs displayed an intermediate yield because they combine the constituents of both leaves, which are rich in metabolites, and stems.

The brown coloration of the stem extract may be associated with the presence of tannins, oxidized compounds, phenolic substances, or other degradation products formed during extraction (Fig. 4) . Conversely, the green color of the leaf and leafy twig extracts likely indicates a high concentration of chlorophylls, plant pigments, and lipophilic compounds associated with leaf tissues. These results further confirm that the leaves are rich in extractable plant metabolites.

Figure 4 Appearance of methanolic extracts obtained from stem, leaves and leafy twigs of *G. phanerophlebia*

3.2. Phytochemical screening results

The table 2 summarizes the results obtained of the phytochemical screening of the methanolic extracts (ST380BTT, ST380BTF, ST380BTRF) from stems, leaves, and leafy twigs of *G. phanerophlebia*.

Table 2 Phytochemical screening results performed on stems, leaves, and leafy twigs of *G. phanerophlebia* extracts

Chemical families	Characterization reagents	ST 380BTT	ST 380BTF	ST 380BTRF
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.

Thus, twenty-four chemical families were investigated in the methanolic extracts obtained from the stems, leaves, and leafy twigs of *G. phanerophlebia* (Table 2). None of the extracts contained all of the screened chemical families. The richest extract was the crude stem extract (ST380BTT), containing 16 chemical families. Next was the crude leafy twig extract (ST 380BTRF), consisting of 15 chemical families. In contrast, the crude leaf extract was the least rich with only 12 identified families.

High amounts of tannins, cardenolides, polyphenols, and coumarins were detected in most of the extracts. Cyanogenic glycosides, saponins, triterpenoids, and anthocyanins were present in low concentration in all three plant parts (stems, leaves, and leafy twigs). None of them contained anthracene glycosides, flavonols, alkaloids, unsaturated sterols or anthracenosides. The abundance of tannins and leucoanthocyanins in all plant parts of *G. phanerophlebia* may justify the traditional medicinal use of this species.

These phytochemical results were analysed using Principal Component Analysis (PCA), which highlighted that the extracts were structured according to their chemical composition (Fig. 5).

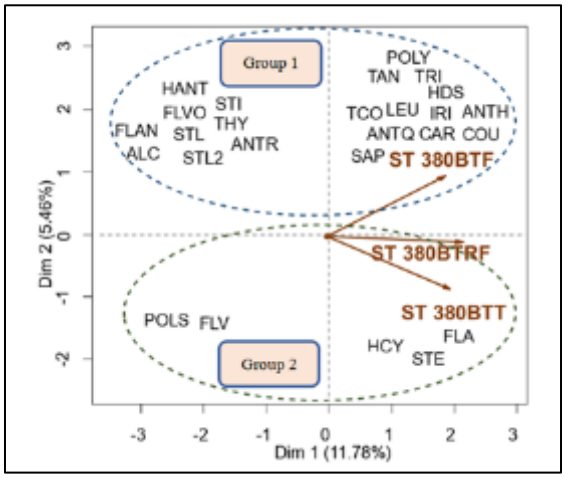

Figure 5 Principal Component Analysis (PCA) of *G. phanerophlebia* stems, leaves, and leafy twigs extracts based on semi-quantitative phytochemical profiles

The factorial map (Dim 1 = 11.78 % ; Dim 2 = 5.46 %) highlights two distinct chemical groups based on metabolite family distribution.

- **Group 1** (upper ellipse), which contain the majority of chemical families, is characterized by high secondary metabolites diversity, including polyphenols (POLY), tannins (TAN), condensed tannins (TCO), anthocyanins

(ANTH), coumarins (COU), leucoanthocyanins (LEU), saponins (SAP), triterpenoids (TRI), iridoids (IRI), cardenolides (CAR), anthraquinones (ANTQ), as well as certain flavonols (FLVO), alkaloids (ALC), and anthracene glycosides (HANT). This reflects a profile rich in bioactive, antioxidant, and potentially pharmacological compounds. The right side of this group is more strongly associated with polyphenols, tannins, and complex glycosidic compounds, while the left side is more closely related to specific flavonoids, alkaloids, and anthracene derivatives.

- **Group 2** (lower ellipse) is characterized by a more restricted composition, dominated by flavones (FLV), flavanes (FLA), steroids (STE), cyanogenic glycosides (HCY), and polysaccharides (POLS). This indicates a profile more oriented toward structural, energetic, or primary defense compounds.

3.2.1. The treatment vectors also show distinct affinities

Leaves extract (ST380BTF) is strongly correlated with phenolic families and complex secondary metabolites such as polyphenols, tannins, anthocyanins, coumarins, saponins. Leafy twigs extract (ST380BTRF) exhibits an intermediate and more generalist profile, while stem extract (ST380BTT) is more closely associated with flavanes, steroids, and cyanogenic glycosides.

Then, stems and leafy twigs extracts showed strong similarity, characterized by a richness in polyphenols, condensed tannins, and cardenolides. In contrast, the leaves extract was distinguished by a significant presence of iridoids and lactonic steroids, indicating notable chemical differentiation.

This phytochemical screening was conducted to identify and compare the likely chemical families present in the different plant parts. However, the results indicate the presence of distinct metabolic profiles, that may be related to physiological, ecological, or anatomical variations among the plant parts studied. This step provides preliminary information about the plant species and helps determine which parts are most promising for future research. It also enabled an estimation of the nature and, to a lesser extent, the abundance of secondary metabolites in the plant powders used to obtain the bioactive extracts. These results may help formulate hypotheses explaining the biological activity of an extract due to the presence of a particular chemical family.

3.3. Antimicrobial activity

3.3.1. Antibiogram results

Table 3 shows the antimicrobial activity of the methanolic extracts from the stems, leaves, and leafy twigs of *G. phanerophlebia* by semi-qualitative evaluation. The activity is expressed as the diameter of the inhibition zone (IZD mm).

Table 3 Inhibition zone diameter of methanolic extracts from the stems, leaves, and leafy twigs of *G. phanerophlebia* against pathogenic microorganisms

Plant part / Microorganisms	**Value of the Inhibition Zone Diameter (IZD mm ± standard deviation)**					
	Stems (ST380BTT)	**Leaves (ST380BTF)**	**Leafy twigs (ST380BTRF)**	**Reference antibiotics**		
				T1	**T2**	**T3**
Gram-negative bacteria						
Escherichia coli	9 ± 0	9 ± 0	7 ± 0	21 ± 0.6	35 ± 0	NT
Salmonella enterica	9 ± 0	6 ± 0	6 ± 0	23 ± 0.3	35 ± 0	NT
Shigella flexnerii	9 ± 0	9 ± 0	6 ± 0	21 ± 0	27 ± 0	NT
Proteus mirabilis	6 ± 0	8 ± 0	8 ± 0	22 ± 0	32 ± 0	NT
Pseudomonas aeruginosa	9 ± 0	12 ± 0	12 ± 0	13±0.6	NT	NT
Yersinia entercolitica	9 ± 0	9 ± 0	6 ± 0	21 ± 0	34 ± 0	NT
Enterobacter cloacae	6 ± 0	6 ± 0	6 ± 0	23 ± 0	10 ± 0	NT
Enterobacter aerogenes	6 ± 0	6 ± 0	6 ± 0	16 ± 0	16 ± 0	NT

Gram-positive bacteria						
Staphylococcus aureus	10 ± 0	10 ± 0	13 ± 0	21 ± 0	23 ± 0	NT
Bacillus cereus	6 ± 0	11 ± 0	11 ± 0	22 ± 0	33 ± 0	NT
Listeria monocytogenes	7 ± 0	9 ± 0	9 ± 0	21 ± 0	27 ± 0	NT
Clostriduim perfringens	6 ± 0	6 ± 0	6 ± 0	21 ± 0.6	26 ± 0	NT
Streptococcus pyogenes	13 ± 0	13 ± 0	15 ± 0	27 ± 0.3	NT	NT
Streptococcus pneumoniae	9 ± 0	10 ± 0	16 ± 0	11 ± 0	26 ± 0	NT
Yeast						
Candida albicans	0 ± 0	0 ± 0	0 ± 0	NT	NT	23 ± 0.6

T1: Neomycin (30µg/disc); T2: Doxycycline (30µg/disc);T3: Miconazole (50µg/disc); NT: Non-Tested

Overall analysis of the antibiogram results shows that the tested extracts exhibit variable antimicrobial activities depending on the part of the plant used, the type of the microorganisms, and the sensitivity of the bacterial strains (Fig.6). The inhibition zones of the plant extracts ranged from 6 to 16 mm. The reference antibiotics showed much larger inhibition zones (10 to 35 mm). No antifungal activity was observed against *Candida albicans*.

3.3.2. Activity against Gram-negative bacteria

The crude extracts from the three parts of *G. phanerophlebia* exhibed moderate sensitivity:

- The stems extract (ST380BTT) presented weak to moderate antibacterial activity, with inhibition zones ranging from 6 to 9 mm for most strains. This low sensitivity may be due to a low concentration of active compounds or to the highly lignified structure of the stems.
- The leaves extract (ST380BTF) manifested slightly better activity, observed against the following strains
- *Pseudomonas aeruginosa* (IZD = 12 mm), *Escherichia coli* and *Shigella flexneri* (IZD = 9 mm).
- The leafy twigs extract (ST380BTRF) demonstrated interesting activity against *P. aeruginosa* (IZD = 12 mm) and *Proteus mirabilis* (IZD = 8 mm).

Thus, Gram-negative bacteria are generally more resistant to the crude extracts from *G. phanerophlebia* stems, leaves and leafy twigs. This may be due to their outer membrane, which is rich in lipopolysaccharides, or their low permeability to bioactive compounds [29]. Nevertheless, some strains such as *P. aeruginosa* showed moderate sensitivity, suggesting the presence of active antibacterial compounds capable of crossing this barrier.

3.3.3. Activity against Gram-positive bacteria

These microorganisms appear to be more sensitive, in general, to the extracts of *G. phanerophlebia* plant parts. The most sensitive strains were *Streptococcus pyogenes* (IZD = 13 mm for stems and leaves ; IZD = 15 mm for leafy twigs), *Streptococcus pneumoniae* (IZD = 16 mm for leafy twigs), *Staphylococcus aureus* (IZD = 13 mm for leafy twigs), and *Bacillus cereus* (IZD = 11 mm for leaves and leafy twigs). Standard antibiotics (Neomycin and Doxycycline) all produced much larger inhibition zones, reaching up to 35 mm for some strains. This indicates that the plant extracts are less potent than conventional antibiotics; however, they still exhibit real and measurable biological activity. In contrast, the reference antifungal agent miconazole showed strong activity (IZD = 23 mm). This suggests that the extracts either do not contain effective antifungal compounds or that their concentrations are insufficient to inhibit this yeast. Since crude extracts are complex mixtures, their activity could potentially be improved by fractionating, purifying, and isolating the active molecules.

3.4. Microdilution results

The following tables quantitatively present the antibacterial activity of the methanolic extracts from the stems, leaves, and leafy twigs of *G. phanerophlebia.* This activity is expressed as the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) against sensitive strains.

3.4.1. Stems extract activity

The table 4 shows the antibacterial activity of the methanolic stem extract of *G. phanerophlebia,* expressed as MIC and MBC values.

Table 4 MIC and MBC values of strains sensitive to the methanolic extracts (ST380BTT) of stems of *G. phanerophlebia*

Extract	Sensitive strains	IZD (mm)	MIC (µg/ml)		MCB (µg/ml)	CMB/CMI ratios	Class
ST380BTT	*Escherichia coli*	9 ± 0	500	M	1,000	2	Bactericidal
	Yesrsinia entercolitica	9 ± 0	>1,000	W	>1,000	ND	-
	Shigella flexnerii	9 ± 0	>1,000	W	>1,000	ND	-
	Staphylococcus aureus	10 ± 0	250	M	>1,000	ND	Bacteriostatic
	Streptococcus pyogenes	13 ± 0	200	M	1,000	5	Bacteriostatic
	Streptococcus pneumoniae	9 ± 0	250	M	>1,000	ND	-
	Pseudomonas aeruginosa	9 ± 0	1,000	W	>1,000	ND	-

ND : Not determined value

These results indicates that *Streptococcus pyogenes* was the most sensitive strain in response to the crude stem extract, followed by *Staphylococcus aureus* and *Streptococcus pneumoniae* (Gram-positive bacteria). However, according to classical criteria, the MBC/MIC ratios rather indicate a bacteriostatic activity . Thus, this while this stem extract can effectively inhibit the growth of *S. pyogenes* at low concentrations, but its lethal effect remains limited. Its antibacterial action may be limited to slowing or stopping bacterial proliferation without destroying the cell completely. In contrast, the MBC/MIC ratio for *Escherichia coli* was 2, indicating a true bactericidal activity. This reveals that the extract can inhibit and subsequently kill bacterial cells at relatively close concentrations. These results suggest the stem extract of *G. phanerophlebia* is effective against this Gram-negative bacterium, despite the moderate inhibition zone.

The high MIC values (>1,000 µg/ml) observed for *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, and *Shigella flexneri* indicate low extract efficacy or even bacterial resistance. This resistance may be related to the organism's highly protective outer membrane or their intrinsic antimicrobial resistance mechanism [30,31]. Overall, the methanolic stem extract exhibits stronger activity against certain Gram-positive bacteria, such as *Streptococcus pyogenes* and *Staphylococcus aureus*, and weaker activity against several Gram-negative bacteria.

3.4.2. Leaves extract activity

The table 5 below presents the antibacterial activity of the methanolic leaf extract of *G. phanerophlebia* expressed in terms of MIC and MBC values.

Table 5 MIC and MBC values of strains sensitive to the methanolic extracts (ST380BTF) from the leaves of *G. phanerophlebia*

Extract	Sensitive strains	IZD (mm)	MIC (µg/ml)		MCB (µg/ml)	CMB/CMI ratios	Class
ST380BTF	*Escherichia coli*	9 ± 0	500	M	1,000	2	Bactericidal
	Yesrsinia entercolitica	9 ± 0	>1,000	W	>1,000	ND	-
	Shigella flexnerii	9 ± 0	>1,000	W	>1,000	ND	
	Staphylococcus aureus	10 ± 0	62.5	E	1,000	16	Bacteristatic
	Streptococcus pyogenes	13 ± 0	250	M	1,000	4	Bactericidal
	Streptococcus pneumoniae	10 ± 0	250	M	500	2	Bactericidal
	Bacillus cereus	11 ± 0	500	M	>1,000	ND	-
	Listeria monocytogenes	9 ± 0	500	M	>1,000	ND	-
	Pseudomonas aeruginosa	9 ± 0	1,000	W	>1,000	ND	-

ND: Not determined value

According to the analysis of this table, the MIC values obtained for the crude leaf extract (ST380BTF) range from 62.5 µg/ml to >1000 µg/ml. This suggests that certain bacteria are more sensitive to the leaf extract than others.

First, Gram-positive bacteria are generally more sensitive than Gram-negative bacteria such as *Streptococcus pyogenes* and *Staphylococcus aureus*. This may be do to the absence of an outer membrane in Gram-positive bacteria,which allows for better penetration of bioactive molecules.

Then, regarding the nature of the antimicrobial activity, the leaf extract exhibits bactericidal activity against *Escherichia coli*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*, and bacteriostatic activity against *Staphylococcus aureus*. These results suggest that the leaves contain compounds capable of either inhibiting bacterial growth or causing bacterial death depending on the strain and concentration.

Globally, the methanolic leaf extract of *G. phanerophlebia* demonstrates intringuing antibacterial activity, especially against Gram-positive bacteria. However, Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, and *Shigella flexneri* were found to be more resistant. These findings suggest that the leaves are a potential source of bioactive antibacterial compounds worthy of further phytochemical investigation.

3.4.3. Leafy twigs extract activity

The table 6 presents the antibacterial activity of the methanolic leafy twigs extract of *G. phanerophlebia* expressed in terms of MIC and MBC values.

Table 6 MIC and MBC values of strains sensitive to the methanolic crude extracts (ST380BTRF) from the leafy twigs of *G. phanerophlebia*

Extract	Sensitive strains	IZD (mm)	MIC (µg/ml)		MCB (µg/ml)	CMB/CMI ratios	Class
ST380BTRF	*Staphylococcus aureus*	13 ± 0	125	E	1,000	8	Bacteriostatic
	Streptococcus pyogenes	15 ± 0	250	M	1,000	4	Bactericidal
	Streptococcus pneumoniae	16 ± 0	250	M	1,000	4	Bactericidal
	Bacillus cereus	11 ± 0	500	M	>1,000	ND	-
	Pseudomonas aeruginosa	12 ± 0	1,000	W	>1,000	ND	-

ND : Not determined value

The analysis of the table reveal moderate antibacterial activity, which is generally higher than that observed with stems and leaves. The MIC values range from 125 to 1,000 µg/ml. The best results were observed against Gram-positive bacteria, particularly *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*, with confirmed bactericidal effects (MBC/MIC = 4). Therefore, the extract is capable of inhibiting bacterial growth and subsequently eliminating bacteria at higher concentrations. These results implay a strong presence of active antibacterial compounds in the leafy twigs. These compounds present in the leafy twigs appear to be particularly effective against Streptococci. This activity may be related to the presence of flavonoids, tannins, or phenolic compounds [32]. Despite the notable inhibition zone (IZD = 12 mm) observed with *Pseudomonas aeruginosa*, the high MIC value (1000 µg/ml) indicates low actual effectiveness. This apparent contradiction may be due to good diffusion of the compounds in the medium but weak intrinsic antibacterial potency. The resistance of *Pseudomonas aeruginosa* is likely related to its highly selective outer membrane, efflux systems, and strong adaptive capacity [33].

In summary, the leafy twigs appear to exhibit the largest inhibition zones and the strongest overall activity compared with stems and leaves alone. This could be due to a higher concentration of secondary metabolites, as well as a possible synergistic effect between the compounds present in the young stems and leaves. The methanolic extract of *G. phanerophlebia* leafy twigs shows moderate antibacterial activity, which is more pronounced than that of stems and leaves combined. *Staphylococcus aureus* was highly sensitive at low concentrations, although the effect was mainly bacteriostatic. Gram-negative bacteria, particularly *Pseudomonas aeruginosa*, were more resistant. These results suggest that leafy twigs are the most promising part of *G. phanerophlebia* for finding natural antibacterial compounds.

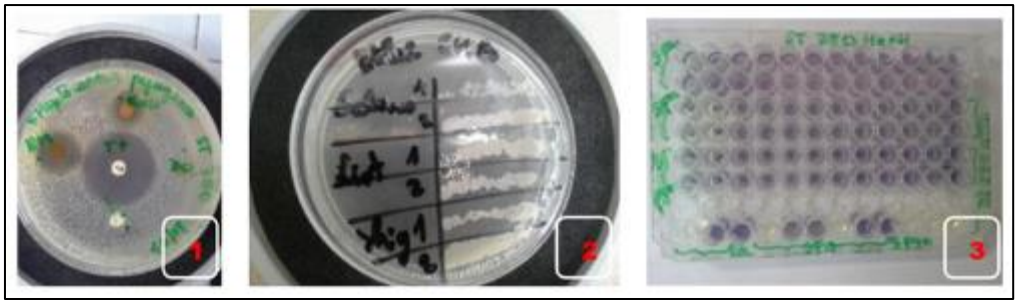

Figure 6 The sensitivity of the strains to the methanolic extracts of *G. phanerophlebia*

3.5. Antioxidant and free radical scavenging activity

The methanolic extracts from the stems, leaves, and leafy twigs exhibited all positive activity, as detected by bioautographic TLC screening (Fig.7). Their free radical scavenging capacity was quantified by determining the IC_{50} value for comparison with that of α-tocopherol. The table 7 below presents an evaluation of the antioxidant activity of the methanolic extracts from the three parts of *G. phanerophlebia* via the DPPH assay.

Table 7 Quantification of the free radical scavenging activities of the methanolic extracts from the stems, leaves, and leafy twigs of *G. phanerophlebia* against DPPH

Plant extract	**TLC-bioautography**	**DPPH inhibition at 50 µg/ml (%)**	**α-tocopherol equivalent (µM/mg/mL of extract)**	**IC_{50} (µg/ml)**
ST380BTT (Stems)	+++	90.20	113.8260	21.27 ± 0.5
ST380BTF (Leaves)	+++	86,24	110.8835	31.77 ± 0.1
ST380BTRF (Leafy twigs)	++++	93.33	548.7714	12.56 ± 0.33
α-tocopherol	+++	93.02	ND	11.43

+++ = highly active banding; ++ = several active bands; + = few active bands; ND = Not determined.

The analysis of these results show that all three extracts have a very strong free radical scavenging capacity, with inhibition percentages above 85 %. The leafy twig extract expressed the greatest activity, with 93.33 % inhibition, even slightly higher than 93.02 % inhibition of α-tocopherol, the antioxidant reference. These results indicates that the leafy twigs are particularly rich in antioxidant compounds. The stem extract shows strong activity (90.20 %), while the leaf extract displays slightly lower but still high activity (86.24 %). Based on the obtained IC_{50} values, the leafy twigs exhibit the best free radical scavenging activity, with an IC_{50} of 12.56 µg/ml. This value is very close to that of α-tocopherol (11.43 µg/ml), indicating remarkable antioxidant activity. The stems show intermediate activity, and the leaves present the weakest activity of the three extracts. Compared to vitamin E activity, the leafy twigs show an extremely high value, approximately five times higher than those of the stems and leaves. These results confirm the leafy twig's very hight antioxidant content and significant biological potential. According to the phytochemical screening results, this activity may be related to the high concentration of flavonoids, phenolic compounds, tannins, and antioxidant terpenes [34]. The strong free radical scavenging potential of the leafy twigs was yet confirmed by TLC bioautography. As shown in the figure 6, the leafy twigs exhibt the highest intensity (+++), indicating the presence of abundant molecules capable of neutralizing DPPH free radicals. These results suggest that *G. phanerophlebia*, particularly its leafy twigs, is a potential source of natural antioxidant compounds that could be utilized in the pharmaceutical and nutraceutical industries.

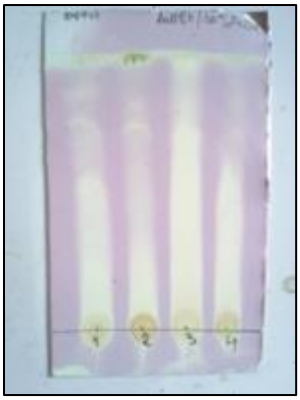

Figure 7 TLC bioautography result of DPPH free radical scavenging

3.6. Anti-plasmodial activity

The table 8 summarizes the 50 % inhibitory concentration values (IC_{50} : µg/ml) of the methanolic extracts from the different parts of *G. phanerophlebia* against the FCM29 strain of *Plasmodium falciparum*.

Table 8 IC_{50} values and antiplasmodial activity of the methanolic extracts from the stems, leaves, and leafy twigs of *G. phanerophlebia* against *Plasmodium falciparum*

***Plasmodium falciparum* (FCM29)**	**IC_{50} : µg/ml of plant extract**			
	ST380BTT (Stems)	**ST380BTF (Leaves)**	**ST380BTRF (Leafy twigs)**	**Quinine (1 mg/ml)**
	25.77 ± 0.02	19.37 ± 0.33	16.63 ± 0.27	0.50 ± 0.1
Class	NA	+	+	++++

NA: Not active, +++ = highly active; + = few active

The IC_{50} value represents the concentration required to inhibit 50 % of parasite growth. The lower the value, the greater the antiplasmodial activity. In this experiment, results show that the leafy twig extract (ST380BTRF) exhibited the greatest activity, with an IC_{50} of 16.63 µg/ml. The leaf extract (ST380BTF) displayed slightly lower activity, with an IC_{50} of 19.37 µg/ml. Finally, the stem extract (ST380BTT) manifested the weakest activity and was considered inactive, with an IC_{50} of 25.77 µg/ml. Quinine at 1mg/ml, used as the reference compound, exhibited a very low IC_{50} value of approximately 0.50 µg/ml, corresponding to very strong antiplasmodial activity. Regarding their antiplasmodial potential, the leafy twig and the leaf extracts were particularly interesting because they exhibited IC_{50} values below 20 µg/ml. According to Kaur *et al*. (2018), an IC_{50} value below 20 µg/mL for a crude extract is often considered promising in phytopharmacology [35]. However, compared with quinine's activity, the studied plant extracts are clearly less potent than the reference antimalarial compound. However, the activities observed in the leaf and leafy twig extracts remain interesting for crude extracts.

This antiplasmodial activity of *G. phanerophlebia*, which belongs to the RUBIACEAE family, may be attributed to the abundance of secondary metabolites such as flavonoids, tannins, terpenes, and phenolic compounds as detected in the crude extracts above [36]. These secondary metabolite families are each known for their antiplasmodial potential, as demonstrated in previous studies [37,38,39,40]. Nevertheless, the leafy twigs were more active than the leaves alone. This may be due to the existence of synergistic effects between the compounds present in the leaves and those in the young stems, or to a potentially higher concentration of certain active constituents in the twigs. It is certain that the biological activities of the extracts are not due to alkaloids or anthocyanins, despite these compounds being well-known for their biological activities. Furthermore, the antiplasmodial result obtained are partially consistent with the previously observed extraction yields. The leaves exhibt the highest extraction yield, the leafy twigs showed an intermediate yield, and the best biological activity. These results indicates that a high extraction yield does not necessarily correspond to greater biological activity, and that efficacy depends primarily on the nature of the extracted compounds rather than solely on their quantity.

4. Conclusion

The comparative chemical and biological study of the three organs of *G. phanerophlebia* revealed that the nature of the plant organ significantly influences the extraction yield, the appearance of the extracts, their chemical composition, and especially their biological and therapeutic activities. The study of the methanolic extracts from the stems, leaves, and leafy twigs of G*. phanerophlebia* revealed significant differences in extraction yield, and in the antiplasmodial, antimicrobial, and antioxidant activities. These variations may originate from the unequal distribution of secondary metabolites within the different plant organs. Among *G. phanerophlebia* species, the leaves contain the digest concentrations extractable substances, followed by the leafy twigs ; the stems contain the lowest concentration. In contrast, the leafy twigs exhibit the strongest antimicrobial, antioxidant, and antiplasmodial activities of the plant. Therefore, the leafy twigs represent the most promising plant material for future phytochemical and biological investigations. The correlations between the phytochemical compounds and the observed biological activities were analyzed. The data collected in this study should provide valuable scientific information on the potential use of *G. phanerophlebia* in developing new raw materials and novel therapeutic, nutraceutical, and cosmetic products.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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