

Evaluation of the Antimicrobial and Anti-Free Radical Scavenging Properties and Phytochemical Analysis of Hydroethanolic Extract of *Phyllanthus muellerianus* (Kuntze) Exell

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Abstract

The aim of this study was to evaluate the antimicrobial and antiradical properties of extracts from the leaves, stems and roots of *Phyllanthus muellerianus*, a plant used in traditional Togolese medicine. Agar well-diffusion and broth microdilution methods were used to assess the antimicrobial potential of hydroethanolic extracts from plant organs. Phytochemical compounds, total phenol and condensed tannin content, and free radical scavenging activity were determined in the three extracts. The results of the antimicrobial tests showed that the extract of *P. muellerianus* leaves was the most active on *Staphylococcus* strains, with inhibition diameters of 17 to 23 mm and minimum inhibitory concentrations (MICs) of between 2.5 and 10 mg/mL. Tannins, saponins, alkaloids and flavonoids were found in all extracts. The *P. muellerianus* leaf extract has 4.23 ± 0.25 mgAGE/g of total phenols, the stem extract has 2.96 ± 0.05 mgCE/g of condensed tannins and the root extract expressed a higher antiradical compounds content (0.125 ± 0.003 mgAAE/g). The results of this study demonstrate the antimicrobial and free radical scavenging potential of the plant and contribute to justify its use in traditional medicine.

Keywords

Phyllanthus muellerianus, Hydroethanolic Extract, Antimicrobial Activity, Phytochemical Compounds, Free Radical Scavenging Power

1. Introduction

Antimicrobial resistance (AMR) is constantly evolution and become a serious public health problem in many countries [1]. Around 1.2 million deaths were recorded in 2019, mainly due to antimicrobial resistance. The picture is bleak, and if no action is taken, the number of deaths will rise to 10 million by 2050 [2]. It seems imperative to find a solution to the resurgence of AMR. Overuse of antibiotics, their availability and failure to comply with dosage are the frequent causes of resistance observed in the various classes of antibiotics [3]. Despite the constant efforts of modern medicine to develop new therapies to eradicate antimicrobial resistance, the problem remains real. What's more, new therapies develop by researchers cost very high to the patient, coupled with side effects that force some patients to neglect their dosage, making treatment difficult. The search for new natural or synthetic molecules is becoming an important task for researchers. Secondary metabolites synthesised by plants could offer an alternative solution for treating microbial infections and improving patient health [3]. These secondary metabolites, such as polyphenols, flavonoids, condensed tannins, saponins and others, have shown very interesting pharmacological properties [4].

Traditional medicine uses plants to treat various illnesses including microbial infections; it exploits the active principles of plants [5]. Before the development of modern medicine, many peoples were already using plants for their dietary, cosmetic and, above all, medicinal virtues [6]. This practice is still used today in many parts of the world, and it is estimated that over 80% of the population use plants for their health needs [7].

People in southern Togo also use plants to treat themselves, and this region is rich in botanical diversity, especially medicinal plants. Several ethnobotanical studies carried out in the south regions of Togo have already listed the different plants used by the population [8] [9]; among the plants listed, we often find *Phyllanthus muellerianus* (Kuntze) Exell, a shrub plant belonging to the *Euphorbiaceae* family [10].

P. muellerianus is used to treat childhood diarrhoea and intestinal disorders; an infusion of the plant's leaves is taken to treat severe dysentery, tetanus and wound infections. The aqueous and methanolic extracts of the leaves and stem bark of *P. muellerianus* were found to have strong antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [11]. The chloroform extract of the leaves showed antifungal activity against *Candida albicans* [12]. Stem bark extract showed antibacterial activity against *E. coli* and *S. aureus* but was inactive against *P. aeruginosa* [13]. Aqueous stem bark extract of *P. muellerianus* has mild inhibitory

activity against *Clostridium sporogenes* ATCC 3584 at a MIC of 900 µg/mL, and is inactive against *S. aureus* ATCC 6538, *Streptococcus mutans* ATCC 21175, *Streptococcus pyogenes* ATCC 19615, *Escherichia coli* ATCC 10536, *Candida albicans* ATCC 10231 [14]. Previous studies have also assessed the antimicrobial [15], anti-inflammatory [16], antioxidant [17] and hepatoprotective [18] activities of extracts from the plant, but these studies remain insufficient. In Togo, this work is very rare or almost non-existent about the specie of the plant present in the region [12]. This study was carried out to assess the antimicrobial and free radical scavenging activities of hydroethanolic extracts of the leaves, stems and roots of *P. muellerianus*, with the goal of providing scientific data that could be used to justify the use of the plant by the population in south of Togo.

2. Material and Methods

2.1. Plant Material

The plant material consisted of the leaves, stems, and roots of *Phyllanthus muellerianus*, collected at Amoussimé in Tabligbo, a village situated about 79 km from Lomé (Togo). The plant was identified and confirmed at the Botany and Plant Ecology Laboratory of the Faculty of Science at the University of Lomé. The organs of the plant are shown in **Figure 1(a)** and **Figure 1(b)**.

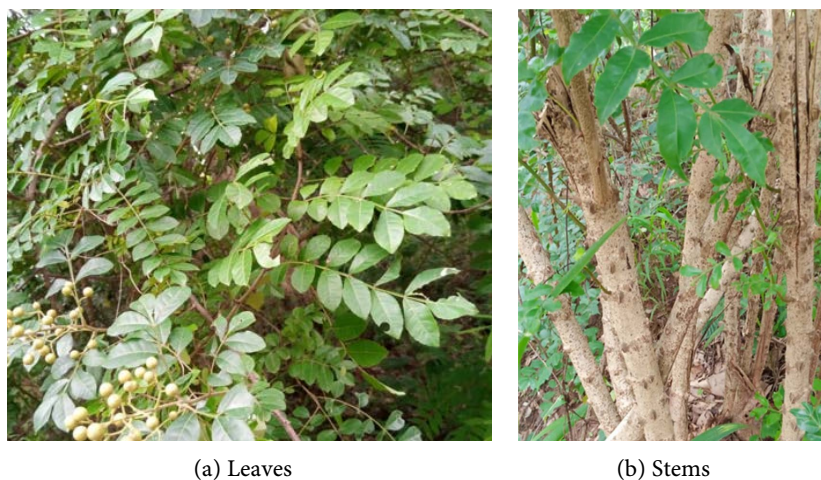


Figure 1. Different part of *Phyllanthus muellerianus*.

2.2. Bacterial Strains

The bacterial strains are made up of clinical and reference strains. The reference strains: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883 were obtained from the bacteriology laboratory of the National institute of hygiene in Lomé. Clinical strains of *S. aureus* (*S. aureus* 0454; *S. aureus* 066; *S. aureus* 064; *S. aureus* 063, *S. aureus* 062; *S. aureus* 056), *Staphylococcus* spp. (*Staphylococcus* spp. 054; *Staphylococcus* spp. 0747; *Staphylococcus* spp. 0746; *Staphylococcus* spp. 048), *Escherichia coli* (*E. coli* 0913; *E. coli* 068; *E. coli* 476), *Pseudomonas*

aeruginosa (*P. aeruginosa* 061; *P. aeruginosa* 065; *P. aeruginosa* 070) and *Klebsiella pneumoniae* (*K. pneumoniae* 0606; *K. pneumoniae* 823; *K. pneumoniae* 452) were obtained from the bacteriology laboratory of the Lomé Campus university hospital. Clinical strains were isolated from urine and pus samples and chosen in this study for their resistance against some reference drugs.

2.3. Extraction

The leaves, stems and roots of *P. muellerianus* were washed and finely cut, then dried in the laboratory under air conditioning for two weeks. The plant organs were then ground to a powder using an electric grinder. For each plant part, hydroethanolic extracts were prepared following the method used by Hoekou *et al.* The hydroethanolic extract was obtained by macerating 50 g of powder in 500 mL of ethanol-water (70/30 (v/v)) 30°C for 48 hours under 300 rpm of stirring. This ethanol-water ratio is better for extracting the polar and nonpolar compounds contained in the plant organ and is compatible with the recommendations of traditional healers. The macerate was filtered through Whatman paper No.1. The various filtrates obtained were evaporated in a Rotavapor (Buchi type) and then freeze-dried to obtain dry extracts [19]. The yield of the different extractions was calculated according to Equation (1):

$$\text{Yield} \left(\text{g} \cdot \frac{100}{\text{g}} \right) = \frac{\text{Weight of dry extract (g)}}{\text{Weight of plant powder (g)}} \times 100 \quad (1)$$

2.4. Preparation of the Microbial Suspension and Extract Solution

The various clinical and reference strains were grown on nutrient agar for 18 - 24 hours. A characteristic colony of each strain was taken by sterile loop and inoculated into 10 mL of sterile physiological water. A turbidity solution equivalent to 0.5 Mc Farland was used to adjust the different microbial suspensions prepared. One gram of each lyophilised extract is dissolved in 10 mL of a sterile ethanol/distilled water mixture to obtain a solution with a concentration of 100 mg/mL, and it was diluted to obtain a concentration of 40 mg/mL. The extracts were sterilised using a syringe filter on 0.22 µm Millipore membrane and verified by culturing an aliquot of this solution on Mueller Hinton agar for 24 hours at 37°C in an incubator.

2.5. Antibiotic Sensitivity of Different Strains

The agar diffusion method with antibiotic-impregnated discs was used. The inoculums were prepared from a pure culture of 18 to 24 hours. To do this, a colony of this pure culture was suspended in 10 mL of saline solution (0.9% NaCl), then the suspension obtained was adjusted by comparison with the 0.5 Mac Farland control. The inoculum thus prepared was used to inoculate Mueller Hinton agar. Antibiotic discs were applied to the agar, which had previously been inoculated with the strain to be tested. After pre-diffusion for 15 minutes at laboratory temperature, the plates were then incubated at 37°C for 18 to 24 hours. The diameter

of the inhibition is measured using a ruler (cm) and then compared with the concordance scale for each antibiotic supplied. This diameter allows to categorise the strains as sensitive (S) or resistant (R) to each antibiotics tested [20].

2.6. Antibacterial Susceptibility Test

The agar well diffusion method was used to assess the antimicrobial properties of different extracts [21] [22]. The prepared microbial suspensions were used to inoculate Mueller Hinton agar by flooding. The tips cut to an internal diameter of 6 mm and then sterilized were used to make wells in the agar. 50 μ L of the different hydroethanolic extracts (40 mg/mL) were deposited in each well. Ethanol-distilled water mixture and gentamicin (5 μ g/mL) were used as negative and positive controls respectively. After the pre-diffusion of the extract, all the plates were then incubated at 37°C for 24 hours. The antibacterial susceptibility was assessed by measuring the inhibition diameters around the wells. All tests were replicated three times.

2.7. Determination of the Minimum Inhibitory Concentration (MIC), the Minimum Bactericidal Concentration (MBC) and the MBC/MIC Ratio

The MIC and MBC were determined using the microdilution technique with Mueller Hinton (MH) broth [22]. A serial dilution of the different hydroethanolic extracts were made from 20 mg/mL to 0.625 mg/mL and 100 μ L of each dilution were dispensed in the microplate containing 100 μ L of the different bacterial strain suspensions. The microplates were incubated at 37°C for 24 hours. The MIC was recorded as the minimum concentration of extract for which no visible growth was observed. The MBC is determined by removing 100 μ L of suspension from wells with no visible growth and inoculating nutrient agar plates that were incubated at 37°C for 24 hours, after which the strain colonies were counted. The MBC/MIC ratio was used to assess the activity of the extract.

2.8. Qualitative Phytochemical

The main phytochemical groups in plant extracts were identified by means of a summary qualitative phytochemical analysis based on colour tests [23]. Tannins, flavonoids, alkaloids and saponins were sought using two methods.

Tannins: Three drops of 1% FeCl₃ solution were added to 1 ml of extract and incubated for two minutes. Or 1 mL of lead acetate solution at 10% was added to 3 mL of extract and incubated for 2 minutes. A blue-black coloration indicates the presence of tannins in two tests.

Flavonoids: Two drops NaOH of 1% solution were added to 1 mL of extract; a positive test was revealed by the presence of a yellow coloration. Or 2 mL of extract are treated with 3 drops of 1% FeCl₃ solution; the positive test is marked by the appearance of a green coloration.

Alkaloids: Two drops of Dragendorff or Mayer reagent are added to 2 mL of

extract. A positive test was marked by a red or white coloration respectively.

Saponins: 1 mL of distilled water was added to 2 mL of aqueous extract, then the solution is stirred for 1 minute. The saponins were confirmed by the presence of a foam that persists for 15 minutes and exceeds 1 cm of height.

2.9. Total Polyphenol (TP) Content

Total polyphenols are measured using the Folin-Ciocalteu Reagent method [24]. The total phenol content is determined by extrapolation on a standard curve obtained from a series of dilutions with distilled water of gallic acid (200 mg/L) ranging from 0.1 to 0.25 mg/mL. A mixture consisting of 0.2 mL of 1 mg/mL extract and 0.5 mL of FCR diluted at half in distilled water was added to the test tubes. After 5 minutes incubation at room temperature, 0.5 mL sodium carbonate (20 g/L) was added to the mixture. The volume in each tube was made up to 4 mL. After shaking, the different solutions were left to stand, protected from light, for 30 min. The optical density was read at 760 nm using a visible UV spectrophotometer (UNICO model 12) against a negative and positive blank. Three readings were taken per sample, and the total phenol content of the various extracts was determined by the following Equation (2).

$$TP = \left(\frac{\text{mgAGE}}{\text{g}} \right) = \frac{(\text{OD} - 0.0218)}{0.2074} \quad (2)$$

where OD is the optical density measured at 760 nm, TP (mgAGE/g) is the equivalent gallic acid concentration per gram (mgAGE/g).

2.10. Condensed Tannin (CT) Content

Condensed tannin content was assessed using the Butanol-HCl method [25]. The reaction leads to the release of anthocyanidins corresponding to the cleaved monomers, which absorb at 540 nm. The test consisted of adding 0.2 mL of ammoniacal iron sulphate (20 g/L) and 7 mL of a butanol/HCl solution (95/5 mL) to 0.2 mL of each extract in tubes. After 30 min incubation in a water bath (Biobase type) at 95 °C, the tubes were cooled and the absorbances were read at 540 nm. The concentration of condensed tannins in the extracts was obtained using the following Equation (3) [26].

$$CT = \left(\frac{\text{mgCE}}{\text{g}} \right) = \frac{(\text{OD} \times 1\text{CE/g})}{0.280} \quad (3)$$

where OD, is optical density measured at 540 nm; 1 CE/g, represent catechin equivalent; CT (mgCE/g) is the catechin equivalent content per gram (mgCE/g).

2.11. Evaluation of Anti-Free Radical Activity (fRA)

Phosphomolybdate reduction was carried out using the method described by Prieto *et al.* (1999); the reaction is based on the reduction of molybdate (VI) to molybdate (V) by the reducing compounds present in the extract after incubation for 90 min at 95 °C. The reduced compound is measured with a spectrophotometer

at 695 nm. Phosphomolybdate (100 mL) was prepared from a mixture of 90 mL 0.6 M sulphuric acid, 5 mL 0.1% sodium phosphate and 5 mL 1% ammonium molybdate. To carry out the test, 1 mL of each extract was added to 9 mL of phosphomolybdate. The whole mixture was heated in a water bath at 95°C for 90 min. Absorbance was measured at 695 nm. Ascorbic acid was used as the standard antioxidant [27]. The ascorbic acid content was determined by the following equation (4).

$$\text{FRA} = \left(\frac{\text{mgAAE}}{\text{g}} \right) = \frac{(\text{OD} \times 0.2222)}{0.0049} \quad (4)$$

where OD is the Optical Density measured at 695 nm; FRA (mgAAE/g) represent the equivalent ascorbic acid concentration per gram (mgAAE/g).

All experiments were performed in triplicate and mean values are presented with standard deviation (mean \pm SD).

3. Results

3.1. Extract Yield

The yield of hydroethanolic extracts from the three plant organs are shown in **Figure 2**. From 50 g of leaf, stem and root powders, the extract yields were 21.99%, 5.94% and 3.66% respectively. The extract from the leaves of *P. muellerianus* showed the highest extraction yield.

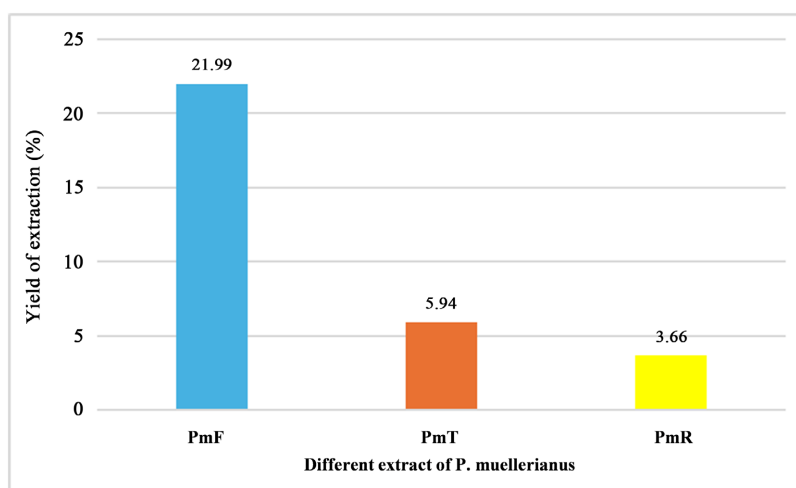


Figure 2. Yield of different hydroethanolic extract of *P. muellerianus*.

3.2. Antibiotic Sensitivity of Different Germs

Reference and clinical strains were tested with various reference antibiotics. The sensitivity profile of the different germs is shown in **Table 1**. Analysis of this table shows that *S. aureus* (n = 6), *Staphylococcus* spp. (n = 4), *Escherichia coli* (n = 3), *Pseudomonas aeruginosa* (n = 3) and *Klebsiella pneumoniae* (n = 3) are resistant to several of the antibiotics tested, including Ampicillin, Amoxicillin, Ticarcillin, Piperacillin and Cefalotin.

Table 1. Antibiogram of different strains.

Drugs tested	Strains								
	<i>S. aureus</i> ATCC 29213	<i>S. aureus</i> (n = 6)	<i>S. spp.</i> (n = 4)	<i>P. aeruginosa</i> ATCC 27853	<i>P. aeruginosa</i> (n = 3)	<i>K. pneumoniae</i> ATCC 13883	<i>K. pneumoniae</i> (n = 3)	<i>E. coli</i> ATCC 25922	<i>E. coli</i> (n = 3)
Ampicillin	S	R (100%)	R (100%)	R	R (100%)	R	R (100%)	S	R (100%)
Amoxicillin	S	R (100%)	R (100%)	R	R (100%)	S	R (100%)	S	R (100%)
Ticarcillin	NT	R (100%)	R (100%)	NT	R (100%)	S	R (100%)	S	R (100%)
Piperacillin	NT	R (100%)	R (100%)	NT	S (66.6%)	NT	R (100%)	S	R (100%)
Cephalothin	S	R (100%)	R (75%)	R	R (100%)	S	R (100%)	S	R (100%)
Cefazoline	S	R (100%)	R (75%)	NT	R (100%)	S	R (100%)	S	R (100%)
Ceftriaxone	S	R (100%)	R (75%)	NT	S (33.3%)	S	R (66.6%)	S	R (66.6%)
Ceftazidime	S	R (100%)	R (100%)	S	R (66.6%)	S	R (66.6%)	S	R (66.6%)
Gentamicin	S	S (80%)	S (100%)	S	R (66.6%)	S	S (100%)	S	R (66%)
Tobramycin	S	S (80%)	S (100%)	S	NT	NT	NT	S	NT
Kanamycin	S	S (60%)	S (100%)	NT	R (100%)	NT	NT	NT	NT
Norfloxacin	S	S (83%)	S (75%)	NT	S (100%)	S	R (66%)	NT	R (66%)
Erythromycin	S	S (83%)	S (75%)	NT	NT	NT	NT	NT	NT
Levofloxacin	S	S (83%)	S (75%)	S	S (100%)	NT	R (66%)	S	R (66%)

NT: not tested, S: sensitive, R: resistant.

3.3. Sensitivity of Germs to Different Extracts

The sensitivity of germs to the different extracts is presented in **Figure 3**. Hydroethanolic extracts of leaves (PmF), stems (PmT) and roots (PmR) were active on *S. aureus* (n = 6), *Staphylococcus* spp. (n = 4) and *S. aureus* ATCC29213 with inhibition diameters ranging from 15 to 23 mm. PmF and PmR were active on *P. aeruginosa* (n = 3) and *P. aeruginosa* ATCC27853 with inhibition diameters ranging from 10 to 15 mm. PmF was active on *K. pneumoniae* ATCC 1388 and *E. coli* ATCC 25922 with inhibition diameters of 10 mm and 11 mm respectively. *K. pneumoniae* (n = 3) and *E. coli* (n = 3) were resistant to the three *P. muellerianus* extracts tested in this study. In the Gram-positive bacteria tested, PmF was more active than PmT, which was also more active than PmR (PmF > PmT > PmR). For the Gram-negative bacteria tested, only PmF and PmT showed activity with *Pseudomonas* strains. In this study, the three hydroethanolic extracts were tested at 40 mg/mL. Gentamicin (reference drug tested at 5 µg/mL), used as a positive control, was active on all germs with inhibition diameters ranging from 10 to 22 mm. The negative control is inactive on all germs.

3.4. MICs, MBCs and Nature of the Activity

In this section, MICs were determined for the hydroethanolic extract of *P. muellerianus* leaves on *Staphylococcus* strains (n = 10); PmF showed better activity than

PmT and PmR, with staphylococci strains. The results are presented in **Table 2**. The MBC and MBC/MIC ratio were also determined. The MIC and MBC values obtained ranged from 2.5 to 10 mg/mL. The best MICs and MBCs were obtained with *Staphylococcus* spp. 048 and *Staphylococcus* spp. 0746 with a value of 2.5 mg/mL. The MBCs determined were the same as the MICs for the extract on the tested germs. The MBC/MIC ratio was therefore unit. The interpretation of this MBC/MIC ratio allows us to conclude that PmF has bactericidal activity on the *Staphylococcus* strains used.

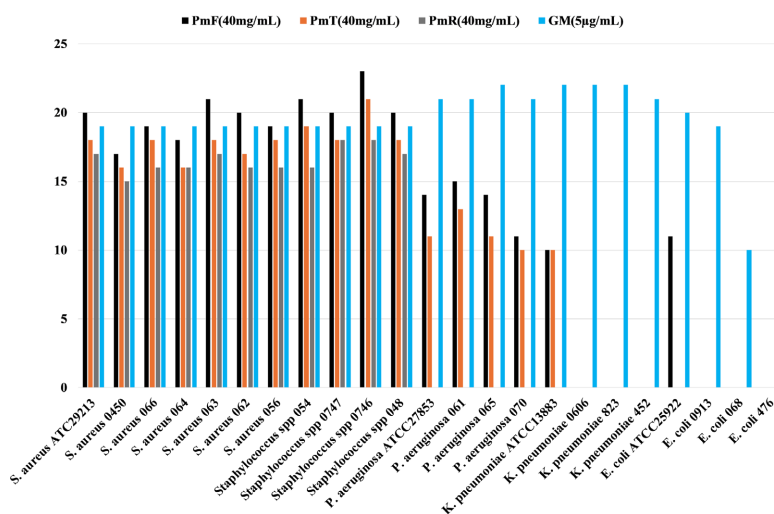


Figure 3. Results of the antimicrobial susceptibility test.

Table 2. MIC, MBC and their ratio of PmF extract.

Strains	PmF extract			Interpretation
	MIC (Mg/mL)	MBC (Mg/mL)	MBC/MIC	
<i>S. aureus</i> ATCC 29213	5	5	1	Bactericidal
<i>S. aureus</i> 0454	5	5	1	Bactericidal
<i>S. aureus</i> 066	5	5	1	Bactericidal
<i>S. aureus</i> 064	10	10	1	Bactericidal
<i>S. aureus</i> 063	5	5	1	Bactericidal
<i>S. aureus</i> 062	5	5	1	Bactericidal
<i>S. aureus</i> 056	5	5	1	Bactericidal
<i>Staphylococcus</i> spp. 054	5	5	1	Bactericidal
<i>Staphylococcus</i> spp. 0747	5	5	1	Bactericidal
<i>Staphylococcus</i> spp. 0746	2.5	2.5	1	Bactericidal
<i>Staphylococcus</i> spp. 048	2.5	2.5	1	Bactericidal

PmF: Hydroethanolic extract of *P. muellerianus* leaves, MIC: minimum inhibition concentration, MBC: minimum bactericidal concentration.

3.5. Qualitative Phytochemistry Analysis of *P. muellerianus* Extract's

The phytochemical study revealed the major phytochemical groups contained in the various extracts of *P. muellerianus*. The tests were carried out in duplicate using two different methods, except in the case of saponins. The results are summarised in **Table 3**. The three extracts contain flavonoids, alkaloids, saponins and tannins.

Table 3. Phytochemical screening of different hydroethanolic extracts of *P. muellerianus*.

Extracts	Alkaloids		Flavonoids		Tannins		Saponins
	Dragendorff	Mayer	NaOH	FeCl ₃	FeCl ₃	Pb(C ₂ H ₃ O ₂) ₂	
PmF	+	+	+	+	+	+	+
PmT	+	+	+	+	+	+	+
PmR	+	+	+	+	+	+	+

+: Presence; -: Absence; PmF: hydroethanolic extract of leaves of *P. muellerianus*; PmT: hydroethanolic extract of stem of *P. muellerianus*; PmR: hydroethanolic extract of root of *P. muellerianus*.

3.6. Total Polyphenolic Content, Condensed Tannins Content and Total Antiradical Activity

The phytochemical screening enabled us to highlight the presence of groups of bioactive molecules. It would be very interesting to measure these compounds and determine their activity. Total polyphenolic and condensed tannins were measured to assess the total anti-free radical activity of these compounds. The results are shown in **Figures 4-6**.

Total polyphenolic in the three extracts was shown in **Figure 4**. PmF extract has a higher content than PmT and PmR, with a value of 4.23 ± 0.25 mgAGE/g. The condensed tannin content (CT) was evaluated in the three extracts and shown in **Figure 5**. The extract of stems of *P. muellerianus* (PmF) has the highest content with a value of 2.97 ± 0.05 mgCE/g. Total anti-free radical activity (fRA) is shown in **Figure 6**. This activity was highest with the root extract (PmR), which was 0.125 ± 0.003 mgAAE/g.

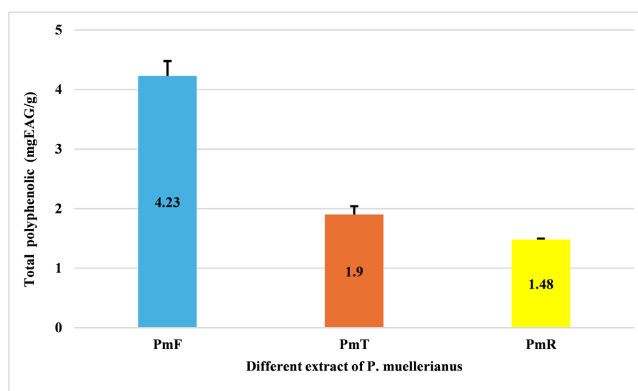


Figure 4. Total polyphenolic (TP) content of different hydroethanolic extract of *P. muellerianus*.

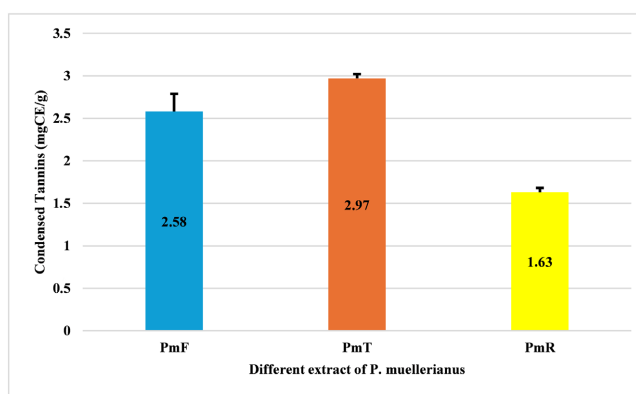


Figure 5. Condensed tannins content (TC) content of different hydroethanolic extract of *P. muellerianus*.

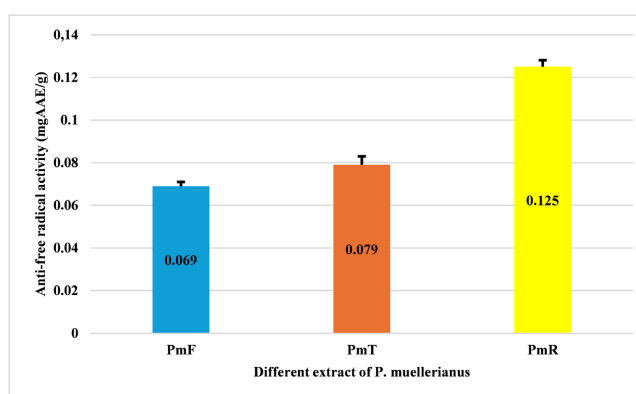


Figure 6. Anti-free radical activity (fRA) of different hydroethanolic extract of *P. muellerianus*.

4. Discussion

The clinical strains chosen in this study are multi-resistant compared to reference strains, and the study wanted to demonstrate whether our plant extracts possess antimicrobial activity against these germs. [28] also tested multi-resistant bacteria against methanolic and aqueous extracts of *P. muellerianus*. Some bacteria have natural resistance to certain groups of antibiotics [29]. The absence of a receptor for the antibiotic, the impermeability of the cell wall, the inactivation of the antibiotic enzyme and the use of efflux pumps associated with the molecule's transporters are the mechanisms involved in bacterial resistance to antibiotics [30].

For the sensitivity of germs to the different extracts, the results of this study were in concordance to those obtained in previous studies. [13] found that *S. aureus* strains with concentrations of 10 mg/mL, 50 mg/mL and 100 mg/mL of ethanolic extract of *P. muellerianus* stem bark had inhibition diameters of 14 mm, 17.5 mm and 20.0 mm respectively. At 50 mg/mL they obtained a diameter of 17.5 mm with stem bark extract from the plant and this value could be inferior to the result of the present study. In fact, PmT tested at 40 mg/mL [40 mg/mL < 50 mg/mL] gave a diameter varying from 16 to 18 with the *S. aureus* strains and moreover we used the whole stem for the preparation of PmT. [28] showed

respective inhibition diameters of 37 mm and 30 mm on *S. aureus* and *P. aeruginosa* with the aqueous leaf extract of *P. muellerianus*. These inhibition diameters were greater than those found on the same bacterial species in this study; their aqueous and methanolic extracts were inactive on strains of *K. pneumoniae* and *E. coli* as well as other germs; the same results were obtained in this study. It should be noted that the study does not specify the concentration at which the aqueous and methanolic extracts were tested. [15] tested aqueous extracts of the leaves and stem and root barks of *P. muellerianus* using the disc method at 8 mg/mL on around twenty clinical and reference strains; they obtained inhibition diameters varying from 7.7 mm to 18.7 mm with good activity on *E. coli* and *K. pneumoniae* strains. These results are higher than those found in this study and the difference could be explained by the extraction and preparation methods for the different extracts and the nature of the strains used. Aqueous extracts of *P. muellerianus* were found to be more active than hydroethanolic and methanolic extracts. [31] [32] tested the extract of *P. muellerianus* leaves alone or in combination with *S. acuta* or *P. amarus* on bacteria at a concentration of 50 mg/mL; the methanolic extracts were purified by solvents of increasing polarity (N-hexane, dichloromethane (DCM), ethyl acetate and butanol). They showed that the ethyl acetate and dichloromethane fractions had a better inhibition diameter than the aqueous fraction and the butanol fraction. 17 mm, 18.7 mm and 22.3 mm were the inhibition diameters obtained on *E. coli*, *S. aureus* and *K. pneumoniae* respectively. These results were superior to the present study and show that fractioning of crude extract into different fractions increases its antimicrobial activity.

The MIC, MBC and MBC/MIC ratio were also determined on the *S. aureus* strains with PmF extract. A MIC of 0.31 mg/mL was found with the methanolic extract of *P. muellerianus* stem bark on *S. aureus* [33]. This value is lower than that found in this study and could be justified by the nature of the solvent and the plant organ used in their study. An MIC of 25 mg/mL and 12.5 mg/mL was obtained in 2021 and 2023 respectively by Obuotor *et al.* on *S. aureus* with an identical MBC of 25 mg/mL, with the extract of ethyl acetate fraction of *P. muellerianus* leaves [31] [32]. These values were higher than those found in this study for the same strains. MICs and MBCs greater than 1000 µg/mL were obtained on *S. aureus* with *P. muellerianus* stem bark extract in previous work by Brusotti *et al.* [14]. Musuasua *et al.* [15] have also shown that *P. muellerianus* extract has bactericidal activity on reference and clinical strains of *S. aureus* with an MIC equal to 2000 µg/mL.

For the phytochemical screening of different hydroethanolic extracts of *P. muellerianus*, the three extracts contain flavonoids, alkaloids, saponins and tannins. Previous work has also shown the presence of these molecules in the leaves and root bark of *P. muellerianus* [12] [28] [31]. The saponins were found absent in the previous study of the plant by [31]; terpenoids and steroids were absent in the methanolic extract but present in the aqueous extract [28]; Polyphenols, alkaloids and anthraquinones were absent in the hydroethanolic extracts of *P. muelle-*

rianus in the study of Olalekan *et al.* [34]. [14] show the presence of polyphenols in these different extracts (aqueous, WE; methanol, ME and fat-free methanol, DME) and an absence of tannins.

Total polyphenolic in the three extracts was shown in the present study. PmF extract has a higher content than PmT and PmR. In terms of polyphenolic, the plant leaves concentrate the molecule the most, followed by the stems and roots. [17] found total polyphenolic values of 39.97 ± 0.07 mg/g tannic acid equivalent on the aqueous extract of the aerial parts of *P. muellerianus*. A value of 10.89 ± 0.17 mg/100g for total phenols was found by [34]; a value of 11.14 mgGAE/g was obtained by [31]. All these values are higher than those obtained in this study. The nature of the extract, the part of the plant used, the methodology and the climatic and soil factors could explain the difference between the results of these authors and the values found in this work. Polyphenols are developed by plants to resist to biotic and abiotic stress conditions; they have biological properties such as antimicrobial, antioxidant and anti-inflammatory [4] [35].

The condensed tannin content (CT) was evaluated in the three extracts. The extract of stems of *P. muellerianus* has the highest content. There are no data in the literature to compare with our values, but a simple determination of the tannins contained in the extract was carried out by [34] with a value of 31.52 ± 2.81 mg/100g. A value of 0.60 ± 0.00 mg/100g with the methanolic extract and a value of 0.75 ± 0.12 mg/100g with the aqueous extract were obtained by [28]. Condensed tannins belong to a subgroup of flavonoids; they were made up of oligomers of flavan-3-ol units and have many interesting biological activities [36]. [37] optimised the determination of condensed tannins in *B. grandis* extract and suggested that methanol and centrifugation were the most significant factors in the extraction of condensed tannins.

Total anti-free radical activity was highest with the root extract (PmR), which was 0.125 ± 0.003 mgAAE/g. This result shown that this extract contained more molecules capable of reducing molybdate (VI) to molybdate (V). A value of 46.69 ± 1.21 mg/100g of total antioxidant capacity was obtained by [34] with *P. muellerianus* leaf extract; a value of 9.15 mg/g was obtained by [31]. These values were higher than the values found in this study. These molecules could be the polyphenols and condensed tannins measured previously, and other compounds revealed by the phytochemical tests. This method assesses the total antioxidant capacity of the extract. This antioxidant capacity decreases in our three extracts as follows: PmR > PmT > PmF.

5. Conclusion

Evaluation of the *in vitro* antimicrobial and anti-free radical scavenging properties of *P. muellerianus* leaf, stem and root extracts showed good activity on the clinical and reference strains used. The leaf hydroalcoholic extract showed the best antimicrobial activity and the highest total polyphenol content; the stem hydroalcoholic extract contained more condensed tannins and the root hydroalcoholic

extract expressed the highest total anti-free radical activity. This study shown that the plant organs tested was endowed with antimicrobial and antiradical activities and contributes to the justification of the use of the plant for the treatment of bacterial infections in the traditional Togolese medicine. Further phytochemical, pharmacological and toxicological studies are required to identify the molecules responsible for these activities and prove the plant's safety.

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Conflicts of Interest

The authors have declared no conflicts of interest.

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