Original Research Article

Phytochemical screening and in vitro antibacterial activity of aqueous extracts of *Phyllanthus muellerianus* (Kuntze) Exell from Kasaï Oriental (DRC) on a few bacterial strains

ABSTRACT

Aims : To carry out a preliminary phytochemical study and to evaluate *in vitro* the antibacterial activities of aqueous extracts of different parts of *Phyllanthus muellerianus*, a plant widely used traditionally in Kasai Oriental to treat various pathologies including several bacteriosis.

Study Design: Based on the results of an ethnobotanical survey in Kasaï Oriental, *P. muellerianus* was selected because of the diversity of recipes involving it, its frequency of citation, its level of preference as well as the diversity of diseases treated and the plebiscite of its effectiveness by traditional healers. To contribute to the enhancement of this plant traditionally used against various bacteriosis and to confirm its therapeutic power, the phytochemical screening of its different parts was carried out and the antibacterial potential of various aqueous extracts was evaluated *in vitro*.

Place and duration of study: Analyzes were carried out from February to September 2017 at the Physico-chemical and microbiological analysis laboratories of the Congolese Office of Control of Mbujimayi as well as at the Biology and Chemistry laboratories of ISP Mbujimayi.

Methodology: Fourteen bioactive chemical groups were investigated in the leaves, stem barks and those of the roots of this plant according to standard methods of characterization based on precipitation, coloring and moss reactions. *In vitro*, the diameters of the zones of inhibition as well as the MIC and MBC of each of different aqueous extracts were determined against 20 bacterial strains of 13 species responsible for bacterial diseases in humans.

Results: The phytochemical screening revealed that all three parts of *P. muellerianus* contain abundant and diverse bioactive chemical groups. This species contains alkaloids, quinones, steroids, terpenes, saponins, tannins, and flavonoids. *In vitro* bacteria-aqueous extract interaction tests demonstrated that all three aqueous extracts have inhibitory activity on several of the 20 bacterial strains tested. According to MIC and MBC, inhibitory performance varies depending on the germs and extracts. The sensitivity of different bacterial strains to an extract is also variable; each extract having its spectrum of inhibitory actions.

Conclusion: The results confirm the therapeutic potential of *P. muellerianus* by the presence of several bioactive substances in all its parts and by the diversity of the inhibitory activity of different extracts on several of a few bacterial strains submitted to the tests.

Keywords : Phyllanthus muellerianus; Kasaï Oriental; phytochemical ; antibacterial activity.

1. INTRODUCTION

In many African countries, traditional medicine is increasingly in demand. The World Health Organization (WHO) says that about 80% of Africans use it to treat or receive treatment [1-2]. For Kalanda, more than 40% of the African population carry out all of their disease treatment procedures within traditional herbal medicine [3]. Following an ethnobotanical survey carried out in the province of Kasaï Oriental in the Democratic Republic of Congo (DRC), Musuasua & al. [4] have identified several medicinal plants among which, *Phyllanthus muellerianus* which is traditionally used to treat various bacterial pathologies.

This *Phyllanthaceae* was cited 697 times by 314 or 80% of 391 traditional healers interviewed who considered it very effective against several bacterial diseases. The frequency of citations of this species (FC = 80%), the level of preference (NP = 76.98%), the index of diversity of recipes or use (IDR = 2.22), as well as the diversity of diseases treated and the plebiscite of its effectiveness make this species a plant highly appreciated by traditional healers in Kasaï Oriental [4-5]. These declarations which attribute curative virtues to this species are known but remain just declarative; are they then true in the absence of any scientific proof?

So the aim of this work is to carry out the phytochemical screening of different parts of *P. muellerianus*, determine *in vitro* the diameters of the zones of inhibition of its aqueous extracts as well as their minimum

inhibitory and bactericidal concentrations on a range of 20 strains (isolates and reference strains) belonging to 13 bacterial species. This in order to give scientific proof of its antibacterial therapeutic potential and promote this widely used in Kasaï Oriental traditional medicine [4].

The interest of this work would reside in the fact that the exploitation of its results and its conclusions could direct phytochemists, pharmacologists and toxicologists on new avenues of research likely to lead to the development of improved traditional drugs accessible to low-income people.

2. MATERIALS AND METHODS

2.1. Material

2.1.1. Analytical framework

This research was carried out over a period of nine months from February to September 2017. The experimental analyzes were carried out in the physico-chemical analysis laboratory and the microbiology laboratory of the Congolese Control Office of Mbuji mayi as well as in the Biology and Chemistry laboratories of ISP Mbujimayi.

2.1.2. Microbiological material

A total of 20 bacterial strains belonging to 13 species responsible for bacterial diseases in humans were tested against aqueous extracts of the plant. Among these bacteria, there were 12 strains of different species isolated from local patients and 8 ATCC reference strains of 8 species (Table 1).

Table 1: Bacterial germs used

N°	Gram-type	Strains of isolates	Reference strains
1.	–	*Citrobacter fruendii*	*Citrobacter fruendii* ATCC 43864
2.	–	*Enterobactér aerogenes*	*Enterobactér aerogenes* ATCC 13048
3.	–	*Escherichia coli*	*Escherichia coli* ATCC 8739
4.	–	*Klebsiella pneumoniae*	*Klebsiella pneumoniae* ATCC BAA-1144
5.	–	*Proteus mirabilis*	-
6.	–	*Proteus vulgaris*	*Proteus vulgaris* ATCC 6380
7.	–	*Pseudomonas aeroginosa*	*Pseudomonas aeroginosa* ATCC 15442
8.	–	*Salmonella typhi*	-
9.	+	*Staphylococcus albus*	-
10.	+	*Staphylococcus aureus*	*Staphylococcus aureus* ATCC 6538P
11.	+	*Streptococcus faecalis*	*Staphylococcus epidermidis* ATCC 12228
12.	+	*Streptocoque du groupe D*	-
TOTALS		**12**	**8**

2.1.3. Plant material

The plant material used in this work consists of leaves, stem barks and roots of *P. muellerianus* (local names: Luangandindi, musangala or musengela). It was harvested on February 2, 2017 on the concession of the Banyi Kandolo Agropastoral Farm in Tshiupula, Miabi Territory in the province of Kasaï Oriental in the DRC. It is a phanerophyte plant of the *Phyllanthaceae* family and quite ubiquitous in Kasaï Oriental. It has the habit of a liana-like shrub with 3 spines curved downwards at each node (Fig 1). It knows a cauliflory and some flowers come out on the petioles of the compound and odd-pinnate leaves. Its buds and young leaves are purplish and turn green as they age [6].

Figure 1: Phyllanthus muellerianus (Photos du 02/02/2017)

2.2. Methods

2.2.1. Treatment of plant material

After harvest, the plant material was directly brought and spread out for drying for 7 to 10 days at the temperature of the dark room of the OCC/Mbujimayi laboratory, which varied from 22°C to 35°C during the day with an average of 28°C for the period concerned However, the stems and roots were, depending on the case, dusted and their bark scraped in the fresh state before drying in order to facilitate subsequent grinding. After drying, each sample was carefully taken up and separately crushed and sieved (sieve with 550 µm mesh) and stored in such a way as to avoid any contamination by the powders of the other parts.

2.2.2. Chemical screening

Qualitative phytochemical screening was performed on infused or extracted powders of different parts of *P. muellerianus*. The main chemical families of bioactive secondary metabolites have been sought by standard protocols [7-8].

2.2.3. Used bacterial strains

The local strains of bacteria tested were obtained from the Bacteriology Department of the ISTM/Mbujimayi Laboratory. They were isolated and identified from bacteriological cultures of pathological products of patients (cuproculture, urine culture, blood culture and sperm culture). The specific identification and the purity of each strain were verified according to the classic principles [11,12] before use in the Microbiology Laboratory of the Congolese Control Office (OCC) Mbujimayi. The reference strains used were provided free of charge by the OCC Kinshasa, Mbujimayi, Lubumbashi and Goma laboratories or purchased from PHARMALAB in Kinshasa.

2.2.4. Antibacterial activity

The qualitative method of diffusion in a solid medium from an impregnated disc was used for antibacterial activity [13-17]. The technique consists of inoculating an agar medium with bacteria to be tested by swabbing and allowing them to interact with an aqueous extract carried by a disc of absorbent paper impregnated with the extract. Observe after incubation and measure any zone of inhibition. The negative control was carried out with an identical disc impregnated with distilled water whereas the positive control was done by a disc of a usual antibiotic (Ampicillin, Gentamicin, Cefixime or Negram) chosen according to the recommendations of the Antibiogram Committee of the French Society of Microbiology as a positive control [17].

The Minimum Inhibitory Concentration (MIC) of an extract on a germ or the lowest concentration of this extract that inhibits the germ was determined by the serial double dilution method as recommended by Bruneton. The Minimum Bactericidal Concentration (MBC) or concentration of the same extract, capable of killing more than 99.9% of the germs of the bacterial inoculum (i.e. less than 0.1% of survivors) after 18 to 24 hours of incubation at 37°C was determined by a subculture of “counting” of revivable germs on Mueller-Hinton agar in all the tubes of the series from the MIC towards the highest concentration of extract [7, 18].

For this study an extract whose MIC was less than or equal to 500 µg/mL as recommended by Rios [22], Archambaud [23] and Lawal [24] was considered active. So, based on their MICs, the extracts were categorized as follows:

1) If the MIC > 500 µg/mL, the extract has weak or insufficient antibacterial activity. It is therefore inactive and the germ resists it.
2) If the MIC ≤ 500 µg/mL but greater than 250 µg/mL, the germ is sensitive and the extract exhibits moderate antibacterial activity;
3) If the MIC ≤ 250 µg/mL but greater than 125 µg/mL, the extract has strong antibacterial activity;
4) Finally, if the MIC ≤ 125 µg/mL, the extract has very strong antibacterial activity.

For determination of the Minimum Bactericidal Concentration (MBC), as recommended by Rios [22] and CA-SFM [17], 100 µL of the contents of each tube remaining macroscopically clear from the MIC tube were collected and inoculated separately according to the enumeration technique to test sterility and/or count revivable bacteria. The concentration of the last tube in which no revivable bacteria were detected or in which it was counted less than 100 cfu/mL after 24 hours of incubation is considered as the MBC of the extract on the concerned germ.

The CMB/CMI ratio makes it possible to characterize the antibacterial action of an extract by determining whether it is bactericidal or bacteriostatic. For this work, the interpretation of the results of the CMB/CMI reports was made according to Moroh's recommendations, which are identical to those of the Antibiogram Committee of the French Society of Microbiology (table 2) [17, 25, 27].

Table 2: Determination of the type of antibacterial activity of the extract

N°	Operation of CMB and CMI	Antibacterial activity of the extract
1	If the ratio $\frac{MBC}{MIC} = 1$	absolute bactericidal
2	If $1 < \frac{MBC}{MIC} \geq \mathbf{4}$	Bactericidal
3	If $4 < \frac{MBC}{MIC} < \mathbf{16}$	Bacteriostatic
4	If $\frac{MBC}{MIC} \geq 16$	Extract tolerance

3. RESULTS AND DISCUSSIONS

3.1. Presentation of the results

3.1.1. The bioactive chemical groups of P. *muellerianus*

Table 3 shows the bioactive chemical groups found in different parts of this plant.

Table 3: Bioactive chemical groups of different parts of P. muellerianus

N°	Bioactive chemical groups	leaves	Stem bark	Root bark
1.	Alkaloids	++	+++	+++
2.	Quinones	+++	+++	++
3.	Tannins	++	+++	+++
4.	Gallic tannins	+++	+++	++
5.	Catechic tannins	+	+	+++
6.	Catechols	-	-	+
7.	Flavones	++	-	-
8.	Flavonols and flavononols	-	++	++
9.	Leucoanthocyans	+++	++	+++
10.	Free genins	++	+	+
11.	Steroids	+	+	+
12.	Terpenes	+	++	++
13.	Saponin index	< 10	10	36.6

According to Table 3, *P. muellerianus* contains alkaloids, quinones, gallic and catechin tannins, leucoanthocyanins, free genins, steroids, terpenes and saponins. The distribution and abundance of different bioactive chemical groups vary between parts. Only the leaves contain flavones while they are devoid of catechols, flavonones and flavonols (and flavononols). Catechols and flavonols (and flavononols) are lacking in the stem barks while the root barks do not contain flavones. This richness in secondary metabolites and their diversity in a single plant would justify the therapeutic properties attributed to this plant against several diseases and would explain the satisfaction of the patients to whom this plant was prescribed as well as their healers.

3.1.2. Diameters of the *in vitro* zones of inhibition of bacteria by different aqueous extracts

The results of the inhibition tests of the bacterial strains tested by the aqueous extracts of *P. muellerianus* are recorded in Table 4.

Table 4: Diameters of zones of inhibition of bacterial strains by different aqueous extracts

Origins of strains	Bacterial strains	Leaves	Bark Stem	Root Barks	Usual antibiotic
Reference strains LIOFILCHEM s. r. L	*C. fruendii* ATCC 43864	12.9	15.4	13.8	21.9
	E. aerogenes ATCC 13048	12.7	13.6	13.2	18.1
	E. coli ATCC 8739	10.2	11.5	10.6	17.5
	Kl. Pneumoniae ATCC BAA-1144	11.3	12.8	12.5	14.1
	P. vulgaris ATCC 6380	16.2	14.7	18.7	25.1
	Ps. aeruginosa ATCC 15442	11.2	12.5	10.6	28.2
	St. aureus ATCC 6538P	13.5	12..3	14.1	26.2
	St. epidermidis ATCC 12228	12.1	11.8	15.1	29.9
ISOLATES or strains of bacteria isolated locally.	*C. fruendii*	13.2	12.5	13.1	30.4
	E.aerogenes	11.0	10.5	13.6	15.9
	E coli	9.5	10.7	11.9	23.3
	Kl. Pneumoniae	9.1	12.1	13.3	14.2
	P mirabilis	16.1	15.5	17.4	18.8
	P vulgaris	17.2	14.6	17.6	18.5
	Ps. aeruginosa	9.3	7.7	12.3	25.3
	Sal typhi	8.5	9.6	11.7	20.7
	S. albus	17.3	11.2	18.4	28.5
	S. aureus	13.4	12.3	17.7	23.4
	Str D	10.8	12.9	18.2	22.5
	Str faecalis	11.9	9.3	15.5	27.4

Table 4 shows that with discs soaked in solutions of 8mg/mL extracts, all three aqueous extracts inhibited most bacterial strains tested in this study. The diameters of the inhibition zones vary from 7.7mm to 18.7mm depending on the germs and depending on the extracts. However, they are lower than the inhibition diameters of the usual antibiotics used.

3.1.3. MIC, MBC and action spectra of different aqueous extracts tested

The *in vitro* evaluation of the performance of the active aqueous extracts made it possible to determine the type of action of the extract on each strain of bacteria and to establish the spectrum of actions of each of the extracts compared to the 20 strains of 13 species bacteria tested (table 5).

Table 5: Antibacterial parameters of aqueous extracts of *P. muellerianus*

Aqueous extracts of	BACTERIAL STRAINS	Inhibition zone diameter	MBC (µg/mL)	MIC (µg/mL)	Ratio CMB/CMI	Types of Action on Bacteria	Activity of the aqueous extract
Sheets	*Proteus mirabilis*	16.1	500	94	5.3	Bacteriostatic	Very strong
	Proteus vulgaris	17.2	500	125	4.0	Bactericidal	Very strong
	P. vulgaris ATCC 6380	16.2	500	125	4.0	Bactericidal	Very strong
	Citrobacter fruendii	13.2	750	188	4.0	Bactericidal	Strong
	Staphylococcus epidermidis ATCC 12228	12.1	1000	188	5.3	Bacteriostatic	Strong
	C. fruendii ATCC 43864	12.9	1000	250	4.0	Bactericidal	Strong
	Staphylococcus albus	17.3	1500	250	6.0	Bacteriostatic	Strong
	Staphylococcus aureus	13.4	2000	375	5.3	Bacteriostatic	Moderate
	Staphylococcus aureus ATCC 6538P	13.5	2000	375	5.3	Bacteriostatic	Moderate
	Streptococcus faecalis	11.9	2000	500	4.0	Bactericidal	Moderate
	E. aerogenes ATCC 13048	12.7	1500	500	3.0	Bactericidal	Moderate
	Enterobacter aerogenes	11.0	1500	500	3.0	Bactericidal	Moderate
	Streptocoque du groupe D	10.8	3000	750	4.0	Bactericidal	Weak
	E. coli ATCC 8739	10.2	2000	750	2.7	Bactericidal	Weak

	Klebsiella pneumoniae	9.1	3000	750	6.0	Bacteriostatic	Weak
	Escherichia coli	9.5	2000	1000	2.0	Bactericidal	Weak
	Pseudomonas aeruginosa ATCC 15442	11.2	3000	1000	3.0	Bactericidal	Weak
	Klebsiella Pneumoniae ATCC BAA-1144	9.3	1500	1000	1.5	Bactericidal	Weak
	Pseudomonas aeruginosa	9.3	4000	1000	4.0	Bactericidal	Weak
	Salmonella typhi	8.5	>8000	2000	-	Non déterminée	Weak
Root bark	*Staphylococcus epidermidis ATCC 12228*	15.1	750	63	12.0	Bacteriostatic	Very strong
	Staphylococcus albus	18.4	1000	94	10.7	Bacteriostatic	Very strong
	Proteus vulgaris	17.6	500	94	5.3	Bacteriostatic	Very strong
	Staphylococcus aureus ATCC 6538P	14.1	1000	94	10.6	Bacteriostatic	Very strong
	P. vulgaris ATCC 6380	18.7	750	125	6.0	Bacteriostatic	Very strong
	Proteus mirabilis	17.4	500	125	4.0	Bactericidal	Very strong
	Citrobacter fruendii	13.1	1000	125	8.0	Bacteriostatic	Very strong
	Streptocoque du groupe D	18.2	1000	188	5.3	Bacteriostatic	Strong
	Streptococcus faecalis	15.3	1000	250	4.0	Bactericidal	Strong
	Enterobacter aerogenes	13.6	1000	250	4.0	Bactericidal	Strong
	Staphylococcus aureus	17.7	3000	375	8.0	Bacteriostatic	Moderate
	C. fruendii ATCC 43864	13.8	1500	375	4.0	Bactericidal	Moderate
	E. aerogenes ATCC 13048	13.2	1500	375	4.0	Bactericidal	Moderate
	Klebsiella pneumoniae	13.3	1500	500	3.0	Bactericidal	Moderate
	Klebsiella Pneumoniae ATCC BAA-1144	12.5	1500	500	3.0	Bactericidal	Moderate
	Pseudomonas aeruginosa	12.3	1500	500	3.0	Bactericidal	Moderate
	Pseudomonas aeruginosa ATCC 15442	10.6	2000	500	4.0	Bactericidal	Moderate
	Escherichia coli	11.9	1500	750	2.0	Bactericidal	Weak
	E. coli ATCC 8739	10.6	2000	750	2.7	Bactericidal	Weak
	Salmonella typhi	11.7	3000	1000	3.0	Bactericidal	Weak
Stem bark	*Staphylococcus epidermidis ATCC 12228*	11.8	1500	94	16.0	tolerant	Very strong
	Proteus mirabilis	15.5	750	250	3.0	Bactericidal	Strong
	Proteus vulgaris	14.6	1000	250	4.0	Bactericidal	Strong
	P. vulgaris ATCC 6380	14.7	750	250	2.7	Bactericidal	Strong
	C. fruendii ATCC 43864	15.4	1000	375	2.7	Bactericidal	Moderate
	Klebsiella Pneumoniae ATCC BAA-1144	12.8	1000	375	2.7	Bactericidal	Moderate
	Citrobacter fruendii	12.5	1500	375	4.0	Bactericidal	Moderate
	Pseudomonas aeruginosa ATCC 15442	12.5	1500	375	4.0	Bactericidal	Moderate
	Pseudomonas aeruginosa	7.7	2000	375	5.3	Bacteriostatic	Moderate
	Staphylococcus aureus	12.3	2000	500	4.0	Bactericidal	Moderate
	Staphylococcus aureus ATCC 6538P	12.3	2000	500	4.0	Bactericidal	Moderate
	Klebsiella pneumoniae	12.1	1500	500	3.0	Bactericidal	Moderate
	Streptocoque du groupe D	12.9	2000	500	4.0	Bactericidal	Moderate
	Staphylococcus albus	11.2	1000	500	2.0	Bactericidal	Moderate
	Enterobacter aerogenes	10.5	2000	750	2.7	Bactericidal	Weak
	E. aerogenes ATCC 13048	13.6	2000	750	2.7	Bactericidal	Weak
	E. coli ATCC 8739	11.5	2000	750	2.7	Bactericidal	Weak
	Escherichia coli	10.7	2000	750	2.7	Bactericidal	Weak
	Streptococcus faecalis	9.3	1500	750	2.0	Bactericidal	Weak
	Salmonella typhi	9.6	4000	1500	2.7	Bactericidal	Weak

This table 5 shows that each aqueous extract of *P. muellerianus* inhibits the 20 bacterial strains tested in this work differently. The best MIC is 63 µg/mL on *S. epidermidis* ATCC 12228 by the aqueous extract of the bark of the roots. This finding suggests that these three extracts would each contain active ingredients that differ either by their nature or by their content.

The analysis of this table reveals that the antibacterial activities of all three extracts are low (MIC > 500 µg/mL) on the two strains of *E. coli* and on that of *S. typhi*. These three strains are then considered insensitive to all these extracts.

The extract from the bark of the roots of *P. muellerianus* is effectively active on all 17 other strains belonging to 11 different species with very strong, strong or moderate actions and bacteriostatic or bactericidal effects depending on the germs. The zones of inhibition observed on three strains of two Gram-negative species (*E. coli, E. coli* ATCC 8739 and *S. typhi*) by this extract are low. These three germs showed intermediate sensitivity, requiring high concentrations of this aqueous extract. For the seven strains of six other different species, the aqueous extract of the root barks of *P. muellerianus* has very strong antibacterial activities with bactericidal effects on the isolate of *P. mirabilis* (MIC = 125 µg/mL) and bacteriostatic on *S. epidermidis* ATCC 12228, *S. albus, S. aureus* ATCC 6538P, *P. vulgaris, P. vulgaris* ATCC 6380 and *C. fruendii* with respective MICs of 63; 94; 94; 94; 125 and 125 µg/ml. On the other hand, the actions of this extract are strong and bactericidal on isolates of *S. faecalis* and *E. aerogenes* (CMI = 250 µg/mL) and strong but bacteriostatic on *Streptococcus D* with a MIC of 188 µg/mL. On the remaining strains, the antibacterial activities of the bark extract of *P. muellerianus* are moderate. They are bacteriostatic with an MIC of 375 µg/mL on the isolate of *S. aureus* whereas they are bactericidal on *C. fruendii* ATCC 43864 and *E. aerogenes* ATCC 13048 (MIC = 375 µg/mL) as well as on *Klebsiella pneumoniae* (isolate and ATCC BAA-1144) and *Pseudomonas aeruginosa* (isolate and ATCC 15442) with an MIC of 500 µg/mL.

In addition to three strains commonly insensitive to all three extracts, the aqueous extract from the stem barks still weakly inhibits the two strains of *Enterobacter aerogenes* and that of *Streptococcus faecalis*. It is therefore only active on 14 strains tested, whereas six strains are insensitive to it. Among the sensitive strains, this extract exerts very strong antibacterial activity on *S. epidermidis* ATCC 12228 (CMI=94 µg/mL), however, this strain proves to be tolerant since its CMB is 1500 µg/mL. This aqueous extract causes strong

and bactericidal inhibitions with an MIC of 250 µg/mL on all the strains of *Proteus* tested and moderate and bacteriostatic antibacterial activities on the isolate of *Pseudomonas aeruginosa* with an MIC of 375 µg/mL. Its inhibitory activities are strong and bactericidal with the same MIC of 375 µg/mL on the two strains of *C. fruendii*, on *K. Pneumoniae* ATCC BAA-1144 and on *P. aeruginosa* ATCC 15442 whereas the MIC is 500 µg/mL for *K. pneumoniae, S. albus, S. aureus, S. aureus* ATCC 6538P and *Streptococcus D*. As for the leaf extract, to the three commonly insensitive strains are added all the strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* as well as that of *Streptococcus D* which makes a total of 8 strains of five different species which are weakly inhibited and therefore considered as insensitive to this extract. Of the 12 remaining strains, the inhibitory activity is very strong on all three strains of the genus *Proteus* (MIC of 94 and 125µg/mL) while being bacteriostatic on *P. mirabilis* and bactericidal on the two strains of *P. vulgaris*. This extract has a strong inhibitory activity with bactericidal effects on the two strains of *C. fruendii* (isolate and ATCC 43864) with respective MICs of 188 and 250 µg/mL against a strong inhibitory activity but with bacteriostatic effects on *Staphylococcus epidermidis* ATCC 12228 and *S. albus* whose MICs are respectively 188 and 250 µg/mL. The five other strains sensitive to this extract are from three different species and show moderate antibacterial activity. These are two strains of *S. aureus* (isolate and ATCC 6538P) on which the extract exerts bacteriostatic effects (MIC of 375 µg/mL) and two strains of *Enterobacter aerogenes* (isolate and ATCC 13048) as well as of that of *Streptococcus faecalis* on which the extract is bactericidal with the MIC of 500 µg/mL.

It appears from the above results that each aqueous extract of *P. muellerianus* has a fairly broad spectrum of bacterial inhibitory activities against 20 strains of 13 bacterial species tested. The spectrum of inhibitory actions of the aqueous extract of the root barks of *P. muellerianus* includes 17 strains of 11 species which are: *P. mirabilis, S. epidermidis* ATCC 12228, *S. albus, S. aureus* ATCC 6538P, *P. vulgaris, P. vulgaris* ATCC 6380, *C. fruendii, S. faecalis, E. aerogenes, Streptococcus D, S. aureus, C. fruendii* ATCC 43864, *E. aerogenes* ATCC 13048, *Klebsiella pneumoniae* (isolate and ATCC BAA-1144) and *Pseudomonas aeruginosa* (isolate and ATCC 15442). The spectrum of inhibitions of the aqueous extract of stem bark is also broad and covers 14 strains of 9 different species which are sensitive to it: *P. mirabilis, S. albus, S. epidermidis* ATCC 12228, *Streptococcus D* and isolates and reference strains of *P. vulgaris, P. aeruginosa, C. fruendii, K. Pneumoniae and S. aureus*. The spectrum of inhibitory activities of the aqueous leaf extract in turn covers the following 12 strains belonging to 8 bacterial species: *P. mirabilis, S. epidermidis* ATCC 12228, *S. albus, Streptococcus faecalis* as well as ATCC isolates and strains of *P. vulgaris, C. fruendii, S. aureus, E. aerogenes*.

3.2. Discussion

Phytochemical screening of different parts of *P. muellerianus* revealed a qualitative and quantitative richness of naturally bioactive substances. This species contains alkaloids, quinones, steroids, terpenes, saponosides, tannins and flavonoids. These results converge with those of Ben-Bala [6] who, by phytochemical tests of the bark of the roots of *P. muellerianus*, had detected alkaloids, flavonoids, tannins and saponosides. He had considered these metabolites responsible for the antibacterial virtues in this plant. Kabongo [21] and Robineau [28] found similar results for a related species, *Phyllanthus niruri*. In his study, Robineau [28] had detected alkaloids, saponins and terpenoids in the extract of this plant, whereas Kabongo [21] had detected on the same species, flavonoids, Steroids and tannins in addition to bioactive group detected by Robineau. The diversity of bioactive chemical groups revealed in *P. muellerianus* justifies the multiple uses and the number of pathologies treated with this plant as well as the satisfaction of users.

The results of the *in vitro* tests carried out in this work demonstrated that all three aqueous extracts of *P. muellerianus* inhibited several of the 20 bacterial strains tested. According to the MICs and the CMBs, the inhibition performances vary according to the germs and according to the extracts. The sensitivity of various bacterial strains to an extract is also variable and each extract has its own spectrum of inhibitory actions on the same range of bacterial strains tested.

The conclusions of this study converge with those reported for the same species collected in Togo. Indeed, in a study aiming to justify the traditional use of four plants of the Togolese flora in the treatment of infantile diarrhoea, Hoekou [29] demonstrated *in vitro* the antibacterial and antifungal potential of hydroethanolic extracts of the leaves of *P. muellerianus* on germs often implicated in childhood diarrheal diseases. These are *Escherichia coli, Shigella flexneri, Salmonella typhi, Salmonella enteritidis* and *Candida albicans* with MICs of the extracts varying from 0.5 to 4 mg/mL.

In the present study, all three extracts had MICs varying from 750 to 2000 µg/mL for the two common species, *E. coli* and *S. typhi* also tested by Hoekou. Although the latter declared these two bacterial species sensitive to the hydroethanolic extract of leaves of this plant with MIC values below 4mg/mL, in the present study they were considered insensitive according to the principle of Rios [22], their MICs to crude aqueous extracts being greater than 500 µg/mL. Indeed, for this author, the sensitivity of a bacterial germ to a crude extract is only acceptable if the MIC is less than or equal to 500 µg/mL. This shows a similarity in the

behavior of these germs to the extracts of this plant species despite the difference in the conclusions following the principles considered.

The similarity of the results of this work with those found by other researchers elsewhere demonstrates the relevance of endogenous knowledge associated with local plant species in the herbal medicine of Kasai Oriental. The fact that several bacterial strains of the pathogenic species have been shown to be sensitive to different extracts of *P. muellerianus* undoubtedly justifies the frequent and regular use of this plant in traditional medicine of Kasaï for the treatment of various bacterial infections. The diversity of inhibited bacterial strains and that of the inhibitory actions manifested by an extract explain and support the multiple ethnopharmacological uses of this plant species against several bacteriosis in Kasaï Oriental.
The diversity, the distribution in the different parts and probably also the variation in the levels of the bioactive substances detected explain on the one hand, the diversity of the actions of different extracts on the same bacterial strain; and on the other hand, the multiple uses as well as the number of pathologies treated. This would also be the basis of the difference in the spectra of inhibitory actions of the three extracts.

All these findings confirm the conclusions from the ethnobotanical survey according to which *P. muellerianus* was classified as one of the plants reputed to be very effective against some bacterial and non-bacterial diseases in Kasaï Oriental [4]. The frequency of citations and the number of recipes show that this species give satisfaction to users and the presence of bioactive substances with therapeutic properties in this plant would be the basis of the plebiscite of efficacy attributed to it.

4. CONCLUSION

Phyllanthus muellerianus is a plant species widely used in herbal medicine in Kasaï Oriental, a province of the DRC. The frequency of use is very high and the bacterial diseases treated are numerous. Statements on the effectiveness of drug recipes containing its various parts given without any scientific proof raise questions about the relevance of the practices concerned. Thus, to enhance this plant and attempt to scientifically justify its therapeutic use, this research was conducted to determine the phytochemical composition of different parts in bioactive metabolites and to evaluate *in vitro*, the antibacterial potential of its different aqueous extracts.
To achieve this, the phytochemical screening looking for fourteen chemical groups of bioactive substances was carried out for the leaves, the barks of the stem and in those of the roots. The *in vitro* evaluation of the antibacterial activities of the aqueous extracts of different parts by determining the diameters of the zones of inhibition as well as the minimal inhibitory and bactericidal concentrations against the 20 bacterial strains of 13 species responsible for bacteriosis subjected to the tests.
The obtained results confirm the therapeutic potential of *P. muellerianus* by the following conclusions:

- All parts of this plant contain several bioactive chemical groups (alkaloids, quinones, steroids, terpenes, saponosides, tannins and flavonoids);
- All three extracts contain bacteria-inhibiting principles;
- Their inhibiting actions differ depending to the germs and the extracts;
- Each of the extracts has its own spectrum of action against 20 bacterial strains tested.

The bioguided fractionations of the extracts of this plant prove to be relevant and likely to provide new curative molecules or to promote the development of an improved traditional drug accessible to low-income households.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

The author **MMM** designed and conducted the study. He wrote the first draft of the manuscript. Authors **KBGM** and **MDB** collaborated in the collection of local bacterial strains and facilitated the performance of the tests in the laboratory, Authors **KON** and **PTM** wrote the protocol and supervised the work. Authors **KLK** and **MDMY** managed the literature searches. All authors have read and approved the final manuscript.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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