Original Research Article

Phytochemical screening and *in vitro* antibacterial activity of the aqueous extract of *Phyllanthus niruri* L. from Kasaï Oriental in DR Congo

ABSTRACT

Objectives: The aim of this work is to carry out a phytochemical study and to evaluate *in vitro* the antibacterial potential of the aqueous extract of *Phyllanthus niruri*, a plant traditionally used in Kasai Oriental (DR Congo) against various bacteriosis and other non-bacterial diseases.

Study design: *P. niruri*, was selected from a list resulting from an ethnobotanical survey carried out in Kasai Oriental because of the number of citations and recipes involving it, the level of preference of the species as well as the diversity of diseases treated and the plebiscite of its effectiveness by local traditional healers. To contribute to the enhancement of this plant traditionally used against various bacteriosis and to confirm its potential antibacterial power, it was subjected to phytochemical screening and its aqueous extract was tested *in vitro* on bacterial strains.

Place and duration of the study: The period of this research went from December 2017 to February 2018. The analyzes were carried out at the physico-chemical and microbiological analysis laboratories of the Congolese Control Office of Mbujimayi (DR Congo) and at the Biology and Chemistry laboratories of the ISP Mbujimayi.

Methodology: The chemical groups of the bioactive substances were sought using the classic methods of characterization in solution by precipitation, coloring and foam reactions. The diameters of the zones of inhibition, MICs and CMBs of the aqueous extract of this plant were determined *in vitro* against 20 bacterial strains subjected to the test.

Results: *P. niruri* contains various bioactive chemical groups. Its aqueous extract showed antibacterial activity *in vitro* against several of 20 tested bacterial strains. According to the MICs and CMBs, the inhibitory action spectrum covers 12 bacterial strains out of the 20 tested.

Conclusion: The results found confirm that *P. niruri* has antibacterial principles and a therapeutic potential by the presence of several bioactive substances and the inhibitory power of its aqueous extract on some bacterial strains tested.

Keywords : Phyllanthus niruri -Kasaï Oriental – Phytotherapy – Bacteriosis – Antibacterial activity.

1. INTRODUCTION

According to the WHO, traditional medicine is increasingly in demand in Africa in general and in the Democratic Republic of the Congo (RDC) in particular [1-2]. It is known that more than 40% of the African population carry out all disease treatment procedures within traditional phytotherapy [3]. An ethnobotanical survey carried out by our research team [4] identified 116 plants traditionally used to treat various bacterial pathologies in Kasai Oriental in DRC. Among these plants is *P. niruri*, a Phyllanthaceae developing in the eco-climatic conditions of Kasaï Oriental and which was cited 463 times by 324 (82.86%) of 391 traditional healers interviewed. The latter consider this species as effective against several diseases specifically or possibly bacterial (example: cystitis, gonorrhea, infectious diarrhea, urinary tract infections, typhoid fever, tuberculosis) and other non-bacterial (diabetes, menstrual bleeding, hepatitis B, Illness chronic kidney disease, amoebiasis, verminosis, schistosomiasis or bilharziasis, oxyuriasis, intestinal worms and malaria, kidney stones).

The frequency of citations of this species (FC = 118.41%), the level of preference (LP = 83%), the index of diversity of recipes or use (IDR = 1.43), as well as the variety of diseases treated and the plebiscite of its effectiveness show that this species is well appreciated by the traditional healers of Kasai Oriental [4-5]. However, these assertions on the curative power of *P. niruri* on various bacteriosis are beautiful but remain all declarative and need scientific proof.

Indeed, the traditional pharmacopoeia of the DR Congo [6] mentions that the methanolic extract of the leaves of *P. niruri* growing in Bas-Congo (western DRC) was active against *S. aureus* and that of the leaves with physiological saline was active against *S. aureus* and *Pasteurella pestis*. In the West African Pharmacopoeia [7], mention is made of the antibacterial properties of certain varieties of this species, while Khan et al. [8] confirms the antigonococcal activity of this plant in Madagascar. Even if it must be admitted that individuals or varieties of the same species do not necessarily have the same chemical composition everywhere in the world due to the difference in their eco-climatic environments, the diversity of bacteriosis traditionally treated with this plant raises questions regarding the therapeutic potential and spectrum of antibacterial activity of *P. niruri* from Kasai Oriental.

Thus, this study is conducted with the aim of scientifically proving the therapeutic potential and antibacterial activities of *P. niruri* harvested in Kasaï Oriental (RDC).This, including some germs responsible for pathologies against which this plant is used. To achieve this objective, the phytochemical screening of the whole plant of *P. niruri* is carried out, the diameters of the zones of possible inhibition and the minimum inhibitory (MIC) and bactericidal (MBC) concentrations of the aqueous extract of this plant are determined *in vitro* on 20 bacterial strains (isolates and reference strains) of 13 different species.

2. MATERIAL ET METHODS

2.1. Material

2.1.1. The analytical framework

The experimental analyzes of this study were carried out from December 2017 to February 2018 at the physico-chemical analysis laboratory and the microbiology laboratory of the Congolese Control Office (OCC) as well as in the Biology and Chemistry laboratories of the ISP in Mbujimayi (Kasai oriental province, DRC).

2.1.2. Microbiological material

In this work, 20 bacterial strains of 13 species responsible for bacteriosis were tested, including 12 strains of different species isolated locally and 8 reference strains of 8 species (Table 1).

Table 1: Bacterial germs used

N°	Gram type	Isolate strains	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 aeruginosa*	*Pseudomonas aeruginosa* ATCC 15442
8.	+	*Streptococcus faecalis*	*Staphylococcus epidermidis* ATCC 12228
9.	–	*Salmonella typhi*	-
10.	+	*Staphylococcus albus*	-
11.	+	*Staphylococcus aureus*	*Staphylococcus aureus* ATCC 6538P
12.	+	*Streptococcus D*	-
TOTALS		**12**	**8**

2.1.3. Plant material

The plant material consisted of the whole plant of *P. niruri* (local names: Kahungahunga, kapungapunga in Tshiluba and kapondo in Songie). It was collected on December 20, 2017 in the district of Professors, commune of Kanshi in Mbujimayi in the province of Kasai Oriental (DRC). *P. niruri* is a fairly ubiquitous Phyllanthaceae in Kasai Oriental. It is a herbaceous, erect, entirely glabrous annual plant, measuring 20 to 60 cm in height. It is ruderal and grows back in the fields. Its flowers with separate sexes are inserted on the branches, solitary in the axils of the leaves. The male flowers are at the bottom of the branch, the female flowers at the top.

2.2. Methods

2.2.1. Treatment of plant material

Once harvested, the plant material was directly dusted and spread out for drying in the dark for 5 to 10 days at the temperature of the dark room of the OCC/Mbujimayi laboratory. This varied between 22°C to 35°C during the day with an average of 27.2°C for the period concerned (Cfr the quality management system

records of the ambient conditions of the Microbiology Laboratory of the OCC MBUJIMAYI). Once dried, a sample was crushed, sieved (sieve with 550 µm mesh) and kept.

2.2.2. Chemical screening

Qualitative phytochemical screening was performed using *P. niruri* powder. The chemical groups of bioactive substances investigated were: alkaloids, tannoids, leucoanthocyanins, flavonoids, steroids, terpenoids, quinones and saponins. They were sought by standard methods of characterization in solution based on coloration, precipitation and foam reactions as described by Bruneton [9] and whose operating modes were inspired by Badiaga [10] and Musuasua et al. [11].

2.2.3. Preparation of crude aqueous extracts

Inspired by the approach recommended by Sule [12] and Ouattara [13], the crude aqueous extract of *P. niruri* was obtained by maceration with magnetic stirring of 100g of dry powder of *P. niruri* on which was poured 1000 mL boiling distilled water. After at least 8 hours of shaking, filter the mixture aseptically through sterilized filter paper under the laminar flow hood. Distribute the filtrate in the valves of sterilized and previously weighed Petri dishes, then evaporate until dry in an oven between 45 to 50°C. The dry residue obtained constituted the crude aqueous extract.

2.2.4. Bacterial strains used

The reference bacterial strains used in this work were obtained free of charge from the OCC Kinshasa, Mbujimayi, Lubumbashi and Goma laboratories or purchased from PHARMALAB in Kinshasa. The strains of bacteria isolated locally were from cuproculture, urine culture, blood culture and bacteriological spermoculture in the Bacteriology Department of the ISTM Mbujimayi Laboratory. Verification of the purity of each strain was carried out according to the classic principles recommended by Carbonnelle [14] and Le Minor [15] before use at the OCC Mbujimayi microbiology laboratory.

2.2.5. Antibacterial effect of the aqueous extract

It is the qualitative method by diffusion in a solid medium from a disc impregnated with extract which was used to detect the antibacterial effect of *P. niruri*. The procedure followed was that used by Musuasua et al. [11] which was inspired by CLSI [16], Tsobou [17] and Voukeng [18]. The negative control was ensured by a disc with distilled water. A CLSI disk of an usual antibiotic (Ampicillin (10µg), Gentamicin (high load, 120 µg), Cefixime (5 µg) or Nalidixic Acid (30 µg) chosen according to the recommendations of the Antibiogram Committee of the French Society of Microbiology constituted the positive control [19-20].

2.2.6. Evaluation of the antibacterial activity of the aqueous extract

It is by determining the critical concentrations of the aqueous extract of *P. niruri* on each of the sensitive germs that the antibacterial activities of the extract were evaluated. The Minimum Inhibitory Concentration (MIC) was determined by the serial double dilution method. To obtain MICs and later MBCs closer to reality, the technique was adapted in this work to bring the concentrations of the series closer on the basis of the recommendations of Baker [21] relating to the significant inhibitory concentrations of the extracts. Indeed, instead of a single series of dilutions to prepare the range of concentrations of the aqueous extract to be tested, two series of parallel dilutions were initiated from two stock solutions of different concentrations as carried out in our previuos work [11]. The first stock solution at 32 mg/mL made it possible to obtain the concentrations as recommended by Bruneton [9] and the second stock solution at 24 mg/mL made it possible to introduce intermediate concentrations throughout the series.

The procedures followed to obtain the series of concentrations, for the preparation of the inoculum, for the inoculation-tests as well as for the reading and interpretation of the results are exactly in line with those used in our previous work [11].

The inoculation of the test tubes was done by aseptically introducing 1 mL of the inoculum (approximately 1.5.108 cfu) into each of the tubes from no. 1 to no. 19. The concentration range of the aqueous extract after inoculation was from 8000 µg/mL in tube no. 1 to 23.4375 µg/mL in tube no. 18 [22]. Tube no. 19 is the blank control. The last two control tubes 20 and 21 intended for the sterility control were not inoculated. At the time of reading, each test tube was compared to the 3 control tubes before concluding. The results were scored as follows:

- If a test tube is cloudy, the result is noted (–) because the extract did not inhibit the growth of bacteria. In other words, the concentration of the antibacterial principles of the extract is lower for it to be active on the germ.

- If, on the other hand, a test tube remains unchanged or clear, then the inoculated bacteria have been inhibited by the extract at this concentration and the result is noted (+).

a. Determination of the Minimum Inhibitory Concentration of an active aqueous extract

On the series of test tubes arranged in ascending order, the MIC of the extract is the concentration of the last tube which remained macroscopically clear to the naked eye [23]. As this study evaluated a crude aqueous extract of a plant, it was only considered active on a germ when the MIC was less than or equal to 500 µg/mL as recommended by Rios [24], Archambaud [25] and Lawal [26]. Thus, depending on the MIC on a strain, the action of the extract are considered as follows:

1) If the MIC > 500 µg/mL, the extract has weak or insufficient antibacterial activity. It is therefore inactive and the germ is resistant to 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.

b. Determination of the Minimum Bactericidal Concentration (MBC) of the aqueous extract

Following the recommendations of Rios [24] and CA-SFM [20], 100 µL of the contents of each macroscopically clear tube from the CMI tube were collected and inoculated according to the counting technique to test sterility and/or count the bacteria that have remained revivable. The concentration of the last tube in which all the bacteria were killed or in which it was counted less than 100 cfu/mL after 24 hours of incubation was considered as the Minimum Bactericidal Concentration (MBC) of the extract on the germ concerned.

c. Determination of the type of antibacterial action of the extract on inhibited bacteria

The CMB/CMI ratio made it possible to characterize the antibacterial action of bactericide or bacteriostatic. Once again for this work which evaluated a crude aqueous extract, the interpretation of the CMB/CMI ratios was made according to the recommendations of Moroh [27] which are identical to those of the Antibiogram Committee of the French Society of Microbiology (table 2) [20, 24].

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 DISCUSSION

3.1. Presentation of results

3.1.1. Phytochemical Screening Results for *P. niruri*

Table 3 presents the bioactive chemical groups found in this plant.

Table 3: Bioactive chemical groups present in *P. niruri*

N°	Bioactive chemical groups	Results
1.	Alkaloids	+
2.	Quinones	-
3.	Tannins	+++
4.	Gallic tannins	(+)
5.	Catechic tannins	+

6.	Catechols	-
7.	Flavones	-
8.	Flavonones	+
9.	Flavonols and flavononols	-
10.	Leucoanthocyanins	+
11.	Free genins	-
12.	Steroids	+
13.	Terpenes	+
14.	Saponin index	25

Reading this table 3, *P. niruri* contains 9 chemical groups of bioactive metabolites out of the 14 sought. It contains alkaloids, tannins (gallic and catechic), flavonones, leucoanthocyanins, steroids, terpenes and saponins. Quinones, catechols, flavones, flavonols (and flavononols) as well as free genins were not detected in this species.

3.1.2. Diameters of the *in vitro* zones of inhibition of bacteria by the aqueous extract of *P. niruri*

The results of the inhibition tests of the bacterial strains subjected to the tests by the aqueous extracts of *P. niruri* are recorded in Table 4.

Table 4: Diameters of the zones of inhibition of bacteria by the aqueous extract of P. niruri

Origins of strains	Bacterial strains	*aqueous extract*	Usual antibiotic
Reference strains	*P. vulgaris* ATCC 6380	16.8	25.1
	S. epidermidis ATCC 12228	14.1	29.9
	C. fruendii ATCC 43864	13.8	21.9
	S. aureus ATCC 6538P	13.4	26.2
	P. aeruginosa ATCC 15442	11.9	28.2
	E. aerogenes ATCC 13048	11.3	18.1
	E. coli ATCC 8739	10.9	17.5
	K. pneumoniae ATCC BAA-1144	6.0	14.1
ISOLATES or strains of bacteria isolated locally	*S. faecalis*	16.3	27.4
	Streptococcus D	16.2	22.5
	P. mirabilis	16.1	18.8
	P. vulgaris	15.1	18.5
	S. aureus	14.1	23.4
	C. fruendii	13.5	30.4
	E. aerogenes	11.6	15.9
	E coli	11.5	23.3
	S. albus	11.5	28.5
	P. aeruginosa	11.4	25.3
	K. pneumoniae	6.0	14.2
	S. typhi	6.0	20.7

Table 4 shows that with discs soaked in a solution of 8mg/mL aqueous extract of *P. niruri*, 17 of the 20 bacterial strains tested in this study were inhibited. The diameters of the inhibition zones vary from 10.9 mm to 16.8 mm depending on the germs. However, they are lower than the inhibition diameters of the usual antibiotics used.

3.1.3. MIC, CMB and action spectra of the aqueous extract of *P. niruri*

The results of the *in vitro* evaluation of the performance of the antibacterial power of the aqueous extract of *P. niruri* on the inhibited germs compared to the 20 strains of 13 bacterial species subjected to the tests are recorded in Table 5.

Tableau 5 : Antibacterial parameters of aqueous extracts of P. niruri

N°	BACTERIAL STRAINS	Inhibition zone diameter	MBC in µg/ml	MIC in µg/ml	Ratio CMB/CMI	Types of Action on Bacteria	Activity of the aqueous extract
1.	***P. vulgaris ATCC 6380***	**16.8**	500	125	4.0	Bactericidal	Very strong
2.	***S. faecalis***	**16.3**	750	250	3.0	Bactericidal	strong
3.	***Streptococcus D***	**16.2**	750	250	3.0	Bactericidal	strong
4.	***P. mirabilis***	**16.1**	750	250	3.0	Bactericidal	strong
5.	***S. albus***	**15.5**	1000	375	2.7	Bactericidal	Moderate
6.	***P. vulgaris***	**15.1**	1000	375	2.7	Bactericidal	Moderate

7.	***S. aureus***	**14.1**	1000	375	2.7	Bactericidal	Moderate
8.	***S. epidermidis ATCC 12228***	**14.1**	1500	500	3.0	Bactericidal	Moderate
9.	***C. fruendii ATCC 43864***	**13.8**	2000	375	5.3	Bacteriostatic	Moderate
10.	***C. fruendii***	**13.5**	1500	500	3.0	Bactericidal	Moderate
11.	***S. aureus ATCC 6538P***	**13.4**	1000	500	2.0	Bactericidal	Moderate
12.	***E. aerogenes ATCC 13048***	**12.3**	2000	500	4.0	Bactericidal	Moderate
13.	***P. aeruginosa ATCC 15442***	**11.9**	2000	750	2.7	Bactericidal	Weak
14.	***E. aerogenes***	**11.6**	2000	750	2.7	Bactericidal	Weak
15.	***E. coli***	**11.5**	2000	750	2.7	Bactericidal	Weak
16.	***P. aeruginosa***	**11.4**	4000	750	5.3	Bacteriostatic	Weak
17.	***E. coli ATCC 8739***	**10.9**	4000	1000	4.0	Bactericidal	Weak
18.	***K. pneumoniae***	**6 (–)**	-	-	-	Not active	None detected
19.	***K. pneumoniae ATCC BAA-1144***	**6 (–)**	-	-	-	Not active	None detected
20.	***S. typhi***	**6 (–)**	-	-	-	Not active	None detected

According to the Table 5, the aqueous extract of *P. niruri* inhibited 17 of 20 bacterial strains tested. The aqueous extract of this plant did not inhibit three bacterial strains (*K. pneumoniae, K. pneumoniae ATCC BAA-1144* and *S. typhi*). The best MIC of this extract is 125 µg/mL on *P. niruri*. On susceptible strains, its antibacterial activity ranges from very strong to weak. Indeed, this extract exerts a weak inhibitory activity requiring concentrations greater than 500 µg of extract per mL on five other strains of three different species (isolate and ATCC of *P. aeruginosa* and *E. coli* as well as the isolate of *E. aerogenes*). Together with the previous three, these eight strains are therefore considered insensitive to the aqueous extract of this plant.
This aqueous extract of *P. niruri* has a frank antibacterial activity on the 12 remaining bacterial strains. Its inhibitory activity is very strong (MIC of 125 µg/mL) with bactericidal effects on *P. vulgaris ATCC 6380* and strong (MIC of 250 µg/mL) and bactericidal on *S. faecalis, Streptococcus D* and *P. mirabilis*. On the other hand, it is moderate (MIC of 375 µg/mL) and bacteriostatic on *C. fruendii ATCC 43864* and bactericidal for *S. albus, S. aureus* and *P. vulgaris* whereas the MIC is 500 µg/mL for the isolate of *C fruendii* and *S. epidermidis ATCC 12228*, *S. aureus ATCC 6538P* and *E. aerogenes ATCC 13048.*
According to these results, the antibacterial action spectrum of the aqueous extract of *P. niruri* therefore covers 12 of the 20 bacterial strains tested in this work, which are: *P. vulgaris ATCC 6380, P. mirabilis, C. fruendii ATCC 43864, S. albus, S. aureus, P. vulgaris, C fruendii, S. epidermidis ATCC 12228, S. aureus ATCC 6538P, E. aerogenes ATCC 13048, S. faecalis* and *Streptococcus D*.
The fact that several bacterial strains of pathogenic species have been shown to be sensitive to different extracts of *P. niruri* undoubtedly justifies the use of this plant in traditional Kasaï's medicine for the treatment of various bacterial infections.

3.2. Discussion

Phytochemical screening of the whole plant of *P. niruri* reveals in this species alkaloids, tannins (gallic and catechic), flavonones, leucoanthocyanins, steroids, terpenes and saponins. These results indicate that in the eco-climatic conditions of Kasaï Oriental (DRC), this plant would contain a qualitative diversity of natural bioactive substances likely to confer therapeutic properties on it. These results converge with those of the traditional pharmacopoeia of the DR Congo [6] which states that *P. niruri* from Bas-Congo contains in its aerial part alkaloids, steroids, terpenoids, flavonoids, saponosides, tannins and other polyphenols, lignans. It also has triterpenes (lupeol), alkaloids, tannins, flavonoids, lignans in its roots. The West African Pharmacopoeia [7] also confirms the presence of alkaloids (securinine and related alkaloids), lignans (eg phyllanthin and hypophyllanthin), tannins, flavonoids (quercetin, rutin), saponins in certain varieties of this species. Kabongo [28] and Robineau [29] found fairly similar results for this species. In the Caribbean pharmacopoeia, Robineau confirms the presence of alkaloids, saponins and terpenoids in the extract of *P. niruri* whereas Kabongo by a chemical screening of individuals of the same species collected in Tshilenge part of the Kasaï Oriental, had revealed the presence of flavonoids, steroids and tannins in addition to bioactive groups detected by Robineau. The various bioactive chemical groups highlighted in *P. niruri* of Kasaï Oriental would explain the satisfaction of the users of this plant in phytotherapy and would justify the medicinal uses on the pathologies treated with this plant. These results also converge with those of Ben-Bala [30] and Musuasua et al. [11] on a related species, *P. muellerianus*. Indeed, by phytochemical tests, Ben-Bala [30] had detected alkaloids, flavonoids, tannins and saponins in the bark of the roots of *P. muellerianus* and had confirmed that these groups of metabolites were responsible for the antibacterial properties in this plant. The study by Musuasua et al. [11] had in turn revealed the existence of alkaloids, quinones, gallic and catechic tannins, leucoanthocyanins, free genins, steroids, terpenes and saponins in the aqueous extracts of leaves, stem bark and of the roots of *P. muellerianus* from Kasai Oriental. And for these authors, the aqueous extracts of this plant had also shown a notable antibacterial activity. Similar bioactive chemical groups are found in *P. niruri* from Kasai Oriental and are likely to confer antibacterial power on it as well. Tests performed *in vitro* on antibacterial activity in this work demonstrated that the aqueous extract of *P. niruri* inhibited 17 of 20 bacterial strains tested. According to the MICs and CMBs, the inhibition performance

varies according to the germs. The sensitivity of various bacterial strains to the extract is also variable. With respect to these 20 bacterial strains tested, the action spectrum of the aqueous extract of this plant only includes 12 strains of 9 species out of the 13 tested. The inhibition of five other strains was found weak and are considered insensitive.

The conclusions of this study confirm those of the West African Pharmacopoeia [7] which reported antibacterial properties of certain *P. niruri* varieties. The same applies to the traditional pharmacopoeia of the DR Congo [6] which reports the antibacterial activity of extracts (methanolic and physiological saline) of the leaves of *P. niruri* from Bas-Congo on *S. aureus* and *Pasteurella pestis* without forgetting then that Khan et al. [8] who demonstrated the antigonococcal activity of this plant in Madagascar.

The diversity of bacterial strains inhibited by the aqueous extract of this plant in this study explains and supports the multiple ethnomedicinal uses of this plant species against several bacteriosis in Kasai Oriental. 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 Kasaian herbal medicine.

These results confirm the conclusions from the ethnobotanical survey that *P. niruri* was one of the plants known to be effective against some bacteriosis and other non-bacterial diseases in Kasai Oriental [4]. The frequency of citations and the number of recipes show that this species brings satisfaction to users and the presence of bioactive substances with therapeutic properties in its composition would be the basis of the plebiscite of efficacy attributed to it.

4. CONCLUSION

P. niruri is a plant species widely used in herbal medicine in Kasaï Oriental, a province of the DRC. Its frequency of use is high and the bacterial diseases treated are numerous. The declarations on the effectiveness of medicinal recipes containing this plant without any scientific proof had raised 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 its phytochemical composition in bioactive metabolites and to evaluate *in vitro*, the antibacterial potential of its aqueous extract.

To achieve this, phytochemical screening seeking fourteen chemical groups of bioactive substances was carried out and the *in vitro* evaluation of the antibacterial activity of the aqueous extract by determining the diameters of the zones of inhibition as well as the minimum inhibitory and bactericidal concentrations against the 20 bacterial strains of 13 species responsible for bacteriosis tested.

The results obtained confirm the therapeutic potential of *P. niruri* by:

- The presence in its chemical composition of several bioactive chemical groups (alkaloids, steroids, terpenes, saponosides, tannins and flavonoids);
- The antibacterial power of its aqueous extract on several bacterial strains with an action spectrum covering 12 strains which are: *P. vulgaris ATCC 6380, P. mirabilis, C. fruendii ATCC 43864, S. albus, S. aureus, P. vulgaris, C fruendii, S. epidermidis ATCC 12228, S. aureus ATCC 6538P, E. aerogenes ATCC 13048, S. faecalis* and *Streptococcus D*.

From the above, this plant is likely to provide new healing molecules. This could allow the development of improved traditional medicines accessible to low-income households.

Work to isolate the molecules responsible for the antibacterial activity of this plant is in progress.

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