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EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF HYDROETHANOLIC AND AQUEOUS EXTRACTS OF *PICRALIMA NITIDA* (*APOCYNACEAE*) ON BACTERIAL ETIOLOGIES OF GASTROENTERITIS

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ABSTRACT

Herbal therapy is an ancient form of medicine. It utilizes entire plants or their parts to prevent or treat various diseases thanks to medicinal properties. Bacterial gastroenteritis contribute significantly to healthcare-related burden. The present investigation aimed at evaluating the antibacterial properties of hydroethanolic and aqueous extracts from *Picralima nitida* leaves, bark, and roots on common bacterial etiologies of gastroenteritis. Extraction was performed by maceration with 70% ethanol and water. It was followed by qualitative and quantitative phytochemical analysis. The antibacterial activity was assessed using the macrodilution method on solid medium against four quinolone-resistant bacterial types that are commonly associated to gastrointestinal infections. Results revealed varying extraction yields, with the highest recorded with the hydroethanolic maceration of leaves (22.2%). Phytochemical screening identified several bioactive compounds, with the highest concentrations in total phenols. Minimum Inhibitory Concentrations (MICs) ranged from 8.3 ± 2.5 through 166.7 ± 44.4) mg/mL. Bactericidal potential was also observed. These findings suggest the potential of hydroethanolic and aqueous extracts of *Picralima nitida* in managing infections caused by quinolone-resistant bacteria. These results may justify at least partially the traditional use of *P. nitida* in herbal medicine and emphasize the importance of policies promoting sustainable production of *Picralima nitida*.

KEYWORDS: *Picralima nitida*, antibacterial activity, gastroenteritis.

INTRODUCTION

Herbal therapy, the oldest kind of medicare known to humans involves the use of entire plant or plant part, for the management of various diseases or to support good health. Natural herbs have been extensively used for the treatment and prevention of various ailments since ancient times. Therefore, research on phytomedication become a priority for the health care system.[1] The traditional health-care system is gaining popularity and is still increasing worldwide due to public curiosity about herbal drugs and its marvelous acceptance for their beneficial properties with the least or no side effects against various challenging health-related problems. A significant portion of the global population, around 60%, uses herbal and conventional remedies as their main disease management tools. This preference varies widely across different countries: approximately 80% of people in Africa, 30-50% in China, 48% in Australia, 70% in Canada, 80% in Germany, 42% in the USA, 39% in Belgium, and 76% in France prefer herbal or alternative medications as their initial choice for treating various health conditions.[1]

Infectious diseases are caused by various organisms like bacteria, viruses, fungi, or protozoans. They may lead to significant loss of life, surpassing combined fatalities from famine, war, accidents, and crimes (Lopez *et al.*, 2006). Among the top ten global causes of death, five are infectious diseases (HIV/AIDS, lower respiratory tract infections, diarrheal diseases, tuberculosis, and malaria). According to the World Health Organization in 2018, infectious diseases accounted for 32% of deaths worldwide, with higher proportions in specific regions

such as 68% in Africa and 83.7% in South-Eastern Asia.[2]

In developing countries, particularly in tropical areas, poverty and disease, including both infectious and non-communicable diseases, are prevalent challenges. People in these regions face daily struggles due to these dual burdens. According to WHO estimates, Africa loses nearly 630 million healthy life years annually to disease, amounting to an economic cost exceeding 2.4 trillion international dollars. Achieving related Sustainable Development Goals could potentially prevent about half of this lost productivity value, approximately 796 $ billion, by 2030. In Cameroon, the economic impact of diarrhea is substantial, with direct costs for patients and indirect costs for health districts totaling approximately 29.4$ million annually. Over the next decade, this burden could reach 145 $ million, placing a heavy financial strain on households.[3] Beyond financial implications, diarrhea brings immense suffering to patients and caregivers, compounding anxiety and discomfort. In a healthcare system where out-of-pocket expenses predominate, rising healthcare costs and reduced productivity due to illness exacerbate the challenges faced by families. Antibiotics are currently the primary treatment tools, yet their effectiveness is threatened by misuse and the rise of resistant bacteria. This dilemma underscores the urgent need in developing nations like Cameroon for alternative therapies. Herbal medicines, widely used by 80% of the population, offer a practical and affordable option, particularly beneficial in rural areas where access to conventional healthcare is limited.[4]

In many works on phytotherapy amongst the interest in *Picralima nitida*, a plant commonly known as "Akuamma" or "Kinkeliba" in Cameroon and belonging to the family *Apocynaceae*, is used since antiquity for its pharmacological properties. It is use to manage fever, hypertension, jaundice, for instance. Researches indicates that extracts or isolated compounds from *Picralima nitida* exhibit analgesic effects (Dapaah. *et al.*, 2016), help lower blood sugar levels (Teugwa et *al.*, 2013), and possess anti-malarial properties (Ndjafang *et al.*, 2023). These findings underscore its broad potential as a valuable resource in phytotherapy, reflecting its rich history and promising future in medicinal research.[5] In the same frame to assess its efficacy against some bacterial strains responsible for gastrointestinal diseases, the present work evaluates the *in vitro* antibacterial potential of this plant's extracts through the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and the MBC/MIC ratio. Ultimately, the goal is to empower policies at developing new antibacterial drugs from *Picralima nitida* and contribute to the prevention and control infectious diseases with improved traditional anti-infective agents.[6]

Description of the study area

The Koung-Khi Division is located in the Western region of Cameroon isn't geographically vast. With an area of only 353 square kilometers, it's a relatively small administrative area within the West Region of Cameroon. The Koung-Khi Division is located on the latitude of 5.33848 and longitude of 10.47453. It is bordered to the North by the Mifi, to the South by the Ndé, to the East by the Noun, and to the West by the Haut-Plateau.[16]

Figure 1: Map of the West Cameroon region.[15]

MATERIAL AND METHODS

Plant material and extracts

Material used in this work consisted of leaves, bark and roots of *P. nitida* was collected on 11 January 2024 between 8:00 and 9:00 am at Batoufam, in the Koung-Khi Division, West Cameroon. Identification was carried out at the National Herbarium of Cameroon (HNC) at number: 1942/SRFK. The leaves, bark, and roots were dried in the shade at room temperature, then pulverized with an electric grinder SHB 3056 Turbo Blender and Grinder to obtain a coarse semi-powder.

- **Preparation of the crude hydroethanolic extracts**

In the present work, the hydroethanolic extracts of the plant were prepared by maceration of 200g of leaves, bark and roots semi-coarse powders at room temperature in 70% ethanol in 1000 ml conical flasks. The mixtures were left to macerate for 48 hours at laboratory temperature. These operations were repeated three times in succession. After filtration on Whatman paper, the filtrates were dried in an oven at 40°C. The result was the crude hydroethanolic extracts of the leaves, bark and roots.

- **Preparation of the crude aqueous extracts**

Extraction was carried out using the method described by Bagre *et al.* (2011).[7] The aqueous extracts were prepared by maceration. To this end, 200 g of powdered leaves, bark and roots were weighed using a KERN® PLS balance (Measuring range 2200g, readability of

0,01g) and cold-macerated for 24 h using 700 ml of distilled water on conical flasks. The different products from each of these preparations were filtered through Hydro® cotton and passed through an oven (40°C) to obtain the dry extracts. The dry residues were then recovered, weighed and conditioned packaged for further study.

The different extraction yields were calculated using the following formula:

$$\text{Yield} = \frac{\text{Mass of extract obtained (g)}}{\text{Mass of extract obtained (g)}} \text{ X } 100$$

The extraction products were then concentrated under reduced pressure to have the necessary crude extracts. All extracts were thereafter kept at 4°C until use for the experiments.

Phytochemical screening

The extracts obtained will be subjected to phytochemical screening to detect the presence of major groups of compounds following the protocols described by Harborne.[8] In this study the plant's extracts were screened:

- **Polyphenol identification test**

To identify polyphenols, ferric chloride ($FeCl_3$) test was performed. In two tubes containing 2 mL extract and 2 mL distilled water respectively, four drops of a 10% dilute iron perchloride solution were added. A greenish coloration indicates the presence of polyphenols.

- **Alkaloid detection test**

A small amount of extract (0.5 g) was dissolved in 1 ml of methanol. Addition of a few drops of sulfuric acid 2% and then a few drops of Mayer reagent was performed. Obtaining a white precipitate or turbidity indicates the presence of alkaloids.

- **Test for saponins**

50mg of extract were added to 5 ml of distilled water. The mixture was heated in a water bath for 5 min. after stirring, the foam index should be at least 2cm.

- **Tannin detection test**

50 mg of extract was added to 5 ml of distilled water. The mixture was heated in a water bath for 5 mins, then filtered after cooling. After, 4 drops of 0.5% iron chloride were added to 2 ml of filtrate. The presence of tannins results in the appearance of black blue (dark blue).

- **Liebermann-Burchard test for triterpenes and steroids**

In a hemolysis tube, a small amount of product is dissolved using an appropriate solvent. To the resulting solution, a few drops of Libermann-Buchard reagents are added (1 ml of concentrated H_2SO_4, 20 ml of acetic anhydride, 50 ml of $CHCl_3$), triterpenes give with the reagent a purplish coloration and sterols a bluish-green coloration.

- **Anthraquinones**

At 5 ml of the aqueous solution of dry extract, 5 ml of ammonia at 10% were added. The appearance of a yellow or orange color indicates the presence of bound quinones and a purplish red color, the presence of free quinones.

- **Glycosides**

50 mg of the extract is dissolved in 1.5 ml of HCL 5%, and this mixture is neutralized by 2.5 ml of NaOH 5%, then filtered after homogenization. Then, the hot Fehling liquor (A and B) test is performed. The appearance of a red-brick precipitate indicates the presence of glucosides.

- **Coumarins**

Add to 5 ml of a methanolic solution of extract, a few drops of 10% potash. The appearance of a color varying from blue to purple yellow reflects the presence of coumarins.

- **Flavonoids**

In a tube, a few ml of the methanolic solution of extract was added, a few drops of a soda solution to 1/10. The yellow-orange coloration characterizes the presence of flavonoids.

Quantitative phytochemical screening

The total phenol content of each *P. nitida* extract was determined by the method of Ramde-Tiendrebeogo *et al.* (2012)[9], the total flavonoid content of each extract determined using the aluminum chloride colorimetric method (Chang et *al.*, 2002)[10] and the total tannin content using the Folin-Ciocalteu method as described by Govindappa *et al.* (2011).[11]

Bacteria strains

Four bacterial types were chosen for their frequent involvement in human gastroenteritis. These included 4 clinical isolates (*Staphylococcus aureus, Proteus mirabilis, Serratia marcescens* and *Escherichia coli* ATC 25922) and 3 reference strains (*Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923). Both the clinical isolates and the reference strains were provided the Laboratory of Microbiology of the Université des Montagnes Teaching Hospital and purified on nutrient agar.

Susceptibility tests

All MIC values throughout the study were assessed with the rapid INT colorimetric assay methods as described by Eloff (1998) with some modifications.[12] All extracts primarily underwent dissolution in DMSO/Mueller Hinton Broth (MHB) to the final concentration of DMSO lower than 2.5%.[13] The resulting mixture was thereafter, added to Mueller Hinton Broth, and serially diluted two folds (in a 96-wells microplate). One hundred microliters (100 µL) of the bacterial inoculum (≈ 1.5×10^6 CFU/ML) prepared in appropriate broth was subsequently added to the preparation.[12,13]

- **Determination of the Minimum Inhibitory Concentration (MIC) by the solid medium macrodilution method**

A volume of 2mL of Mueller Hinton agar was introduced into a series of twelve (12) tubes for each extract. They were sterilized at 121°C for 15 minutes in an autoclave. After sterilization, a 2ml volume of each stock solution of each extract was introduced into the tubes containing the agar. 2 ml of the stock solution of the extract was introduced into the Mueller Hinton supercooling agar with a cascade dilution of reason 2. All handling was carried out with the tubes incubated in a water bath at 37°C which allowed to maintain the supercooling agars.

The tubes were then removed from the water bath and tilted at 45° to obtain a base and a slope, then kept for 24 hours at room temperature to ensure dehydration as recommended in the principle of antibiogram by agar dilution. Then seeding was carried out using a platinum loop. To do this, 10 µL of the inoculum was taken to sow on the agar by streaks on the slope. The preparations were then incubated at 37°C for 24 hours. Each test was repeated three times for reproducibility of the test and to obtain a standard value. All assays were performed in triplicate.

- **Determination of the Minimum Bactericidal Concentration (MBC)**

The Minimum Bactericidal Concentration (MBC) corresponds to the lowest concentration of the extracts capable of killing more than 99.99% of the initial bacterial inoculum (or at least 0.01% of survivors). It defines the bactericidal effect of a substance. To determine the CMB during the present work, in 1ml of previously sterilized Mueller Hinton broth, some of the finely scraped agars where no visible growth was recorded were introduced. They were incubated again at 37°C for 24 hours. At the end of the incubation time, growth was materialized by the turbidity of the medium. The MBC was considered from the first tube inside which there was no turbidity at the end of this time. All assays were performed in triplicate.

- **Evaluation of the MBC/MIC ratio**

This report defined the bacteriostatic or bactericidal character of a substance. A ratio greater than or equal to 4 indicates that the substance is bacteriostatic; if this ratio is less than 4, the substance is called bactericidal. If it is equal to 1, then it is called «absolute bactericide».

RESULTS

This work aimed at evaluating the antibacterial activity of hydroethanolic and aqueous extracts of leaves, bark and roots of *Picralima nitida* on bacteria responsible for gastroenteritis. It has generated results that will be presented sequentially.

- **Yields and features of the extractions**

Concerning the yields and features of the different extractions data collected are presented as shown in Table I:

Table I: Yields and physical aspects of the aqueous and hydroethanolic extractions of Picralima nitida

Extracts	Initial masses of powders (g)	Extracts' masses (g)	Percentage yield (%)	Organoleptic features	Scent
AEL	200	20.9	10.5	Dark brownish paste	Savory and herby
AEB	200	23.1	11.6	Dark brownish paste	Savory and herby
AER	200	15.6	7.8	Dark brownish paste	Savory and herby
HEL	200	44.3	22.2	Dark greenish paste	Savory and herby
HEB	200	32.7	16.4	Dark brownish paste	Savory and herby
HER	200	32.4	16.2	Dark brownish paste	Savory and herby

AEL: Aqueous leaves extract; **AEB:** Aqueous bark extract; **AER:** Aqueous roots extract; **HEL**: Hydroethanolic leaves extract; **HEB:** Hydroethanolic bark extract; **HER:** Hydroethanolic roots extract

The information highlighted from these results documented that; In aqueous medium, there was no significant difference in yield between the leaf and the bark (Leaf: 10.5% and Bark: 11.6%) whereas there was a significant difference nearly double in the yield between the aqueous extracts and the hydroethanolic extracts. Nearly 8.2% yield difference was observed globally for all the parts. With the hydroethanolic solvent there was no significant yield difference between the bark and the roots (Bark: 16.4% and Roots: 16.2%). However, the extract yield of the leaves was higher than the other parts.

- **Quantitative and qualitative phytochemical screening**

Regarding the phytochemical screening of the different groups of metabolites, results are found in table II below:

Table II: Qualitative phytochemical composition of the crude extract.

	Aqueous extracts			Hydroethanolic extracts		
Study	Leaves	Bark	Roots	Leaves	Bark	Roots
Alkaloid	+++	+++	+++	+++	+++	+++
Phenolic compounds	++	++	++	+++	+++	+++
Terpenoids	-	-	-	-	-	-

Steroids	-	-	-	-	-	-
Tannins	+++	++	+	++	+	++
Glycosides	+++	+	+++	++	++	++
Anthraquinones	+++	++	+++	++	++	++
Saponins	+++	+++	+++	+++	+++	++
Flavonoids	-	-	+	++	+	+
Coumarins	+	+	+	+	+	+

+++: Very abundant; **++: present**; **+: trace**; **–: Negative**

The result indicates that hydroethanolic extracts contained 80% of the phytochemical groups that were sought, while aqueous extracts contained 70% of the phytochemical groups. Flavonoids were present in the hydroethanolic extracts and aqueous root extracts but absent in the other aqueous extracts.

- **Quantitative phytochemical screening**

Table III displays the finding related to quantification of target substrates

Table III: Quantitative phytochemical screening of *P. nitida* extracts.

Extracts	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	TTC (mg TAE/g extract)
AEL	34.0 ± 0.4	0.8 ± 0,2	19.3 ± 0.7
AEB	17.7 ± 0.6	0	7.7 ± 0.4
AER	28.6 ± 0.7	3.7 ± 0.2	12.1 ± 0.6
HEL	37.3 ± 0.1	20.6 ± 0.5	10.8 ± 0.6
HEB	17.7 ± 0.3	4.7 ± 0.6	1.5 ± 0.4
HER	29.3 ± 0.3	7.4 ± 0.8	6.2 ± 0.3

AEL: Aqueous leaves extracts; **AEB:** Aqueous bark extracts; **AER:** Aqueous roots extracts; **HEL:** Hydroethanolic leaves extracts; **HEB:** Hydroethanolic bark extracts; **HER:** Hydroethanolic roots extracts; **TPC:** Total phenol content; **TFC:** Total flavonoid content; **TTC:** Total tannin content; **GAE:** Gallic acid equivalent; **QE:** Quercetin equivalent; **TAE:** Tannic acid equivalent.

Quantitative analysis of the pharmacologically important phytochemicals in the extracts indicated 94.4% constituents were present in varying amounts in the leaves, bark and roots. The quantity of all the phytochemicals was particularly high in leaves extracts: 37.3 mg GAE/g extract for the TPC, 20.6 mg QE/g extract for the TFC and 19.3 mg TAE/g extract for the TTC. The phytochemical family with the highest quantity was phenol followed by tannins and finally flavonoids as shown in the table.

- **Minimum inhibitory, minimum bactericidal concentrations and the ratio of tested extracts**

The results of the search for different minimum inhibitory concentrations, minimum bactericidal concentrations and the ratio MIC/MBC of tested extracts are recorded in the following table:

Table IV: MIC, MBC, MBC/MIC ratio of hydroethanolic and aqueous extracts of leaves, bark and roots of Picralima nitida on the bacterial strains used.

Bacterial strain	AEL			AEB			AER			HEL			HEB			HER		
	MIC (mg/mL)	MBC (mg/mL)	MBC/ MIC	MIC (mg/mL)	MBC (mg/mL)	MBC/ MIC	MIC (mg/mL)	MBC (mg/mL)	MBC/ MIC	MIC (mg/mL)	MBC (mg/mL)	MBC/ MIC	MIC (mg/mL)	MBC (mg/mL)	MBC/ MIC	MIC (mg/mL)	MBC n(mg/ mL)	MBC/ MIC
SA	83.3±23.6	200	2	166.7±44.4	200	1	166.7±44.4	200	1	20.8±5.9	50	2	20.8±5.9	50	2	20.8±5.9	50	2
SM	83.3±23.6	200	2	166.7±44.4	200	1	83.3±23.6	200	2	16.7±5.9	12.5	0.75	58.3±11.8	50	0.85	16.7±5.9	12.5	0.75
EC	41.7±11.8	100	2	75±10	200	2	41.7±11.8	100	2	25±0	50	2	50±0	100	2	33.3±11.8	50	1
PM	41.7±11.8	100	2	41.7±11.1	100	2	16.7±5.9	50	4	8.3±2.5	12.5	1	12.5±0	25	2	16.7±5.9	12.5	0.75

AE: Aqueous extracts; **HE:** Hydroethanolic extract; **AEL:** Aqueous leaves extract; **HEL:** Hydroethanolic leaves extract; **AEB:** Aqueous bark extract; **HEB:** Hydroethanolic bark extract; **AER:** Aqueous root extract; **HER:** Hydroethanolic root extract; **SA:** *Staphylococcus aureus*; **SM:** *Serratia marcescens*; **EC:** *Escherichia coli*; **PM:** *Proteus mirabilis*; **MIC:** Minimum inhibitory concentration; **MBC:** Minimum bactericidal concentration

To different degrees, hydroethanolic and aqueous extracts acted on all strains studied. Hydroethanolic extracts are the most effective followed by the aqueous extracts. Regarding the hydroethanolic extracts, the lowest MIC (8.3 ± 2.5 mg/mL) was observed with the leaves on *Proteus mirabilis*; while the largest (58.3 ± 11.8 mg/mL) with the back extract on *Serratia marcescens*. For aqueous extracts, the lowest MICs (16.7

± 5.9 and 41.7 ± 11.8 mg/mL) were observed with roots and leaves extracts respectively for only two strains: *Proteus mirabilis* and *Escherichia coli*. By contrast, the MIC obtained for the remaining strains ranged from (75 ± 0 to 166.7 ± 44.4 mg/mL) with this extract. By comparing the MICs obtained with the hydroethanolic extracts and those obtained with the aqueous extracts, we notice that hydroethanolic extracts are 7 times more effective than the aqueous extracts on *S. aureus*, 3 times more on *S. marcescens* and finally 2 times more on the other 2 strains.

DISCUSSION

The present study focused on the antibacterial activity of hydroethanolic and aqueous extracts of *Picralima nitida* on bacteria that cause gastroenteritis.

Plant material consisted of leaves, bark and roots because these parts would have high concentration of secondary metabolites with antibacterial potential.[14] This extraction method conducted at room temperature allows maximum extraction of compounds and prevents their denaturation or modification due to high temperatures.[15]

Thus, to carry out this work, the extraction from the dry powder of the leaves, bark and roots was carried out in solvents of different polarities namely distilled water, and ethanol/water mixture in the proportions 70/30 (v/v). The extraction yields varied from 7.8% to 22.2% for the extracts obtained by aqueous maceration and by hydroethanolic maceration. Hydroethanolic maceration with the best yields attest to its higher extraction power compared to aqueous maceration. The difference in yield observed between total aqueous extracts and total hydroethanolic extracts could be caused by the broad extraction power of ethanol. Water is a solvent that extracts small groups of chemical compounds mainly small and medium molecules and ethanol concentrates many small, medium and large active principles (like flavonoids, phenolic compounds and quinones). The results are in agreement with those from the literature and can be further explained by the fact that the use of solvents with different polarities separates compounds from the crude extract according to their degree of solvent solubility. Also, according to Bourgou *et al.* (2016), the solvent extraction power significantly influences the yield while mixed solvents are particularly effective.[16] In addition to these reasons, the extraction duration could play a role in the difference observed between these extracts, especially because hydroethanolic extracts were left to macerate longer (48 hours) while, aqueous extracts were left for a shorter length of time (24 hours).

Phytochemical screening of the extracts of *P. nitida* revealed that it contained several classes of secondary metabolites namely alkaloids, phenolic compounds, tannins, saponins, flavonoids, glycosides, anthraquinones and coumarins; while terpenoids and steroids were absent. Similar results have been reported by other researchers on *P. nitida* peel and seeds (Obasi *et al.*, 2012 and Solomon *et al.*, 2014). However, there were variations observed in the present work since no terpenoids nor steroids were found.[17,18] This result aligns with the study of Nnamdi *et al.* (2015) whose aqueous extract of the fruit of *P. nitida* did not contain terpenoids.[19] Ngaïssona *et al.* (2016) found flavonoids in their aqueous extracts of the peel, bark and root of *P. nitida*.[20] Also, the aqueous extracts of the leaves and bark in the present investigation did not contain flavonoids. These contrasts may be explained by the fact that, the presence of these secondary metabolites in *Picralima nitida* may differ from one geographic area to the other in connection with ecological determinants linked to inherent biodiversity, pedology, climate, and modifiers that result from human activities. The total phenols, total flavonoids and total tannins contents had concentrations varying from 11.8 through 37.3 mg GAE/g, 0 through 20.6 mg QE/g, and 1.5 through 19.3 mg TAE/g of extract, respectively. These results are different from those obtained by Olumese *et al.* (2023) in a study aiming at investigating the nutritional composition, phytochemical analysis, and antioxidant capacity of ethanol extract of *P. nitida* fruit, bark and pulp using standard methods. The quantitative phytochemical analysis revealed that the total phenols, total flavonoids and total tannins contents were 92.5 mg GAE/g, 331.6 mg QE/g and 64.9 mg TAE/g of extract, respectively. Based on their work which higher levels of secondary metabolites was observed, it could be supported that the synthesis and accumulation of secondary metabolites in plants varies according to biotic and abiotic factors determinants as observed earlier.[3] Biotic factors include the part of the plant used, the age of the plant at harvest or the stage of development, while abiotic factors include climate, season, time and place of harvest, application of fertilizers, and culture density.

Concerning the assessment of the antibacterial activity, the hydroethanolic extracts had the highest MIC, which was obtained with the bark extract on *S. marcescens* at (58.3 ± 11.8mg/mL), while the lowest MIC was obtained with the leaves extract on *P. mirabilis* at (8.3 ± 2.5 mg/mL). In a study at determining drug interactions on multi-drugs-resistant organisms that cause urogenital infections in Cameroon, Tabouguia *et al.* (2018) used the bark of *P. nitida* in various extractions including distilled water, ethanol 50% and ethanol 80%. They tested several bacterial types including *Proteus* spp., *E. coli* and *S. aureus*, and found that the MIC values ranged from 16 through 32 mg/Ml.[21] The smallest MIC of the hydroethanolic extracts in the present research is therefore 2 times more powerful than those observed by Tabouguia *et al.* and would suggest that extracts from the leaves could be as effective or even more effective than the bark. The zone of harvest associated with human activities (Yaoundé for Tabouguia and Batoufam for this work) may at least partially justify the differences as observed above. Moreover, their plant was found not to have most of the screened phytochemicals, implying that

the poor antimicrobial activity displayed depend on the types and concentrations of active components (secondary metabolites) the plant contain.

With a glance on the aqueous extracts, the lowest MICs (16.7 ± 5.9 and 41.7 ± 11.8mg/mL) were observed with the leaves and the roots on *P. mirabilis* and *E. coli.* Oluwakemi *et al.* (2024) investigated the *in vitro* antioxidant and antimicrobial potentials of aqueous extract of *Picralima nitida* seeds and found that, the extract had no effect on *E. coli* whereas, *S. aureus* was inhibited at 30 mg/mL.[22] This could be explained by the fact that seeds may not be as effective as the other parts or also because some of the active metabolites were absent. However, this finding further implies that other parts of *P. nitida* may be useful for the management of some infectious diseases. By contrast, the MIC obtained for the other subjected isolates ranged from 75 ± 0mg/mL through 166.7 ± 44.4 mg/mL with these extracts. In Nigeria Nkere and Iroegbu (2005) investigated antibacterial activity of the root, seed and stembark extracts of *P. nitida* in which extractions were performed by maceration in ethanol, benzene, chloroform, and water. Bacterial strains tested included Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*). Upon completion, the overall MIC value they recorded with the aqueous extracts was 50 mg/mL.[23] This may be explained by the fact that, flavonoids, terpenoids and steroids they contained have higher antibacterial potential. Otherwise, the absence of one or all of these could reduce the antibacterial activity of the extract.

By comparing the MICs obtained with the hydroethanolic extracts and those obtained with the aqueous extracts, it comes out that the hydroethanolic extracts are 7 times more effective on *S. aureus*, 3 times more on *S. marcescens* and finally 2 times more on the others. The activity is, therefore, recorded on both bacterial type (Gram-positive and Gram-negative), but with varying concentrations. The MBC/MIC ratios were between 0.75 and 2 for hydroethanolic extracts and between 1 and 4 for aqueous extracts. Thus, in 95.8% of the cases in total, the action of the two main extracts was bactericidal. The leaves used in this study were then, highlighted as a valuable resource for medication production due to their abundance in antibacterial metabolites and ease of access without damage to the plant.

According to the fact that *E. coli* ATC 25922 is recommended as a surrogate for a broad range of bacteria genera, the findings globally suggest that the MIC values recorded through this work could be a promising way to anticipate the antibacterial potential on the wide range of *Enterobacteriaceae* that are etiologies of gastroenteritis including *Salmonella, Shigella,* and pathogenic *E. coli.*

Plant metabolites have been reported to have several mechanisms just like conventional antibiotics that are used in the management of infectious diseases. The action of phenolic compounds may be due to the sequestration of substrates necessary for growth microbial by chelation of metals such as iron or inhibition of metabolism as sulphonamides and cyclins do.[24] Alkaloids have been reported to have numerous mechanisms including function inhibition of bacterial nucleic acid together with coumarins, inhibition of protein synthesis (macrolides and aminoglycosides), cell wall damage (polymyxin antibiotics) and in addition to the well-established antibacterial mechanisms, alkaloids can also inhibit the activity of bacterial functional proteases and affect DNA topoisomerase and respiration like quinolones.[25] Alkaloids can also be effectively against multidrug-resistant (MDR) bacteria that use efflux pumps to deplete intracellular antibacterial levels, thus preventing bacterial resistance and improving the antibacterial effects of the extract.[25] As a natural phenolic compound, tannins display an antibacterial activity but in addition to this, they may shorten the duration of diarrheal episodes by inhibiting the release of autocoids that cause irritation and inflammation of the intestinal mucosa and leads to the release of prostaglandins which ultimately stimulate the motility and secretion.[26]

CONCLUSION

The evaluation of the antibacterial potential of hydroethanolic and aqueous leaves, bark and roots of *Picralima nitida* on bacterial etiologies of gastroenteritis revealed that these extracts have, at different degrees, bactericidal activity on all strains tested. Hydroethanolic extracts were the most effective with bactericidal effects on all the isolates subjected, while the aqueous extracts were less active. Activity on bacteria expressing different resistance phenotypes would mean that the metabolites contained in *P. nitida*, individually or in combination, could act on mechanisms that are not susceptible to the action of conventional antibiotics alone. Hydroethanolic and aqueous extracts could be used to manage infections due to bacteria resistant to certain antibiotics. Thus, these preliminary results support the folkloric claims of the use of *P. nitida* as herbal remedy. To prevent the rapid selection of resistant strains investigating parameters like doses, administration frequency should be next priorities.

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