ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Investigation on traditional use of *Pericopsis (afrormosia) laxiflora* (Benth.) stem bark in treatment of infectious diseases caused by *Staphylococcus aureus* and *Shigella* sp., two multi-resistants bacteria.

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ABSTRACT

The aim of the present study was to investigate phytochemical constituents of various extracts of *Pericopsis laxiflora* stem bark and their antibacterial activity against *Staphylococcus aureus* and *Shigella* sp., two multi-resistants germs. Preliminary antibacterial activity of aqueous, methanol, ethanol and ethyl acetate extracts was evaluated by agar well diffusion method. Minimum Inhibitory Concentration (MIC) was determined by tube dilution whilst Minimum Bactericidal Concentration (MBC) was determined by agar diffusion method. Aqueous extract have shown lowest activity against the two strains tested with diameters of inhibition (16-17 mm). Whereas, the three organic extracts have recorded the highest activity with diameters of inhibitions (20-22 mm). Aqueous extract gave a MBC of 100 mg/ml on the two germs. Organic extracts were given MBC included between 0.39 and 3.12 mg/ml. Phytochemical screening of all extractsshowed the presence of tannins, flavonoids, steroids andterpnoids, cardiac glycosides, saponins, phenoliccompounds, alkaloid and reducing compounds in different proportions, justifying the differences in their respective antibacterial activities. The study confirms the use of *P. laxiflora* in traditional medicine for treatment of some infectious diseases.

Keywords: Antibacterial activity, *Pericopsis laxiflora*, *Staphylococcus aureus, Shigella* sp., multi-resistants.

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ISSN: 0975-8585

INTRODUCTION

The emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious diseases [1, 2]. This situation creates a serious public health problem both in the developing countries where they are the main cause of high mortality rates, as in the industrialized countries or the resistance to existing antibiotics are being developed at an alarming rate [3].

This observation generates an ever-increasing need to find new antimicrobial compounds to fight these scourges [4]. Also, the scientific world has always made efforts to discover many treatments to reduce the number of diseases caused by these germs. Accordingly, the antimicrobial properties of plants have been studied by many researchers in the world [5,6].Medicinal plants constitute a potential source of this type of compounds [3]. Our objective is to test the antibacterial properties of *P. laxiflora*. The genus *Pericopsis* (syn *Afromosia*) (*Papilionaceae*) is represented by four species: three in Africa and one in Asia [7]. We have *P. laxiflora*, *P. elata*, *P. angolensis* and *P.mooniana*. *P. laxiflora* is a most common species in dry savannah areas while *P. elata*is found in the forest zone of West Africa [8,9].Species of *Pericopsis* are also commonly used in African traditional medicine. *P .laxiflora* bark is often used to cure snake bites, rheumatism, joint pains and teething pains in children [10]. In Côte d'Ivoire, *P. laxiflora* is used to the traditional treatment of many infections: headache, stomach ulcers, stomach aches, upset stomach, gastritis, enteritis, heart pain, abdominal pain [11]. It is used against shigellosis and colibacillosis in Guinea [12]. This plant is used in the treatment of malaria in Ghana [13] and as antiulcer ancestral area Benoue [14] in Nigeria.

In order to achieve our objective, extracts from the bark of *P. laxiflora* were tested on two strains multi-resistant namely *Staphylococcus aureus* and *Shigella sp*.

MATERIALS AND METHODS

Plant material

Some freshly stem barks of *P.laxiflora* was collected in January 2012 in northem Côte d'Ivoire precisely in Lataha a village located 8 km far from Korhogo. The plant was authenticated by the National Floristic Center (NFC) of Felix Houphouët-Boigny University of Cocody, Abidjan where a voucher specimen was deposited.

Bacterial stains

The bacteria used for biological tests are *Staphylococcus aureus* (*S. aureus* n°204C12) and *Shigella sp.* (n°1177C10). These bacterial strains were provided by department of bacteriology and virology at Pasteur Institute of Côte d'Ivoire (PICI). The profiles of these bacteria are showed in table 1.

Table 1: Bacterial germs studied

Bacterial strains	Collected from	Antibacterial profils
S. aureus 204C12	Pus	$CFX^{R}TM^{R}$ GM^{S} TE^{S} CIP^{R} CHL^{R} $COT^{S}PEN^{R}LIN^{S}$ ER^{R} OX^{R} KN^{R} PRI^{S} VAN^{S}
Shigella sp. 1177C10	blood	$CFX^{S}TM^{S}$ GM^{S} TE^{R} CIP^{S} CHL^{R} $COT^{R}AMX^{R}$ AAC^{S} $TCC^{S}TIC^{R}$ TZP^{R} CTX^{R} IPM^{S} AN^{S} NET^{S} CS^{S}

CFX : Cefoxitin, TM : Tobramycin, GM : Gentamicin, TE : Tetracyclin, CIP : Ciprofloxacin, CHL : Chloramphenicol, COT : Cotrimoxazole, PEN : Penicillin G, LIN : Lincomycin, ER : Erythromycin, OX : Oxacillin, KN : Kanamycin, PRI : Pristinamycin, VAN : Vancomycin, AMX : Amoxicillin, AAC : Amoxicillin + ClavulanicAcid, TCC :Ticarcillin + ClavulanicAcid, TIC : Ticarcillin, TZP : Piperacillin, CTX : Cefotaxim, IPM : Imipenem, AN : Amikacin, NET : Netilmicin, CS : Colistin, S : Sensitive, R : Resistant

Preparation of extracts

The stem barks of *P .laxiflora* collected were washed, cut up, and have been dried shelter from sun light for two weeks and reduced to powder by a grinder IKAMAG-RCT type. According to methods described by Guede-Guina*et al.*[15], 100 g of plant powder have been macerated in 1 L of distilled water and homogenized

under magnetic agitation for 24 hours at 25 °C with a agitator IKAMAG-RCT type. The homogenate obtained has been filtered successively two times through hydrophilic cotton (cotton wool) and whatman paper n°2. The volume of filtrate obtained is first reduced with a rotavapor Büchi at 60°C. Then, the remaining of filtrate is evaporated with a Med Center Venticell drying oven at 50°C to provide a grayish powder which is aqueous extract (Etaq).

The same process was carried out by using ethanol, methanol or ethyl acetate instead of distilled water to obtain respectively ethanol extract (Eeth), methanol extract (Emet) or ethyl acetate extract (Eace) [16,17]. All plant extracts obtained are kept in refrigerator till they are used for antibacterial tests.

Antibacterial activity of different extracts

For each bacterial strain, inoculum was prepared by homogenizing 0.1 mL of a suspension opalescent 3 hours in 10mL of Mueller-Hinton broth concentrate twice in order to obtain a bacterial load estimated at 5.10^6 CFU/mL. Also, arange of concentrations from 0.39 to 100 mg/mL was prepared by the method of double dilution [18] for each sample tested.

The susceptibility tests have been carried out on Mueller-Hinton agar (Biorad, France) by using well methods [19,20]. So, like in the case of classic antibiogram realization, every well or hole (6 mm of diameter) has been filled with 80 µL of a 200 mg/mL concentration extract by taken care to separate two holes from at least 20 mm. A reference well has been carried out for each bacterial strain with a 80 µL mixing DMSO solution/sterilized distilled water in proportion 0.5: 0.5 (V/V) [21]. After a 45 minute prediffusion at ambient temperature under hood, the plates have been incubated in an oven at 37°C for 18 to 24 hours. After that period, the extracts action is appreciated by measuring a zone of inhibition (absence of colonies) around the wells.

The antibacterial parameters (MIC and MBC) have been obtained by introducing into a series of hemolysis tubes numbered from T_1 to T_{10} 1 mL of bacterial inoculums. Then, 1 mL of plant extract with a known concentration according to the range of prepared concentrations has been added in the same tubes. That sharing out of plant extracts has been done so that 1 mL of plant extract of 100 mg/mL may be transferred to the tube T_1 that of 50 mg/mL to tube T_2 and so on, and so forth till to tube T_9 that will receive 1 mL of plant extract of 0.39 mg/mL. The tube T_{10} received instead of plant extract, 1 mL of DMSO/distilled water (1/13: V/V) which been use as reference. That plant extract preparation with a known concentration in each of the tubes containing previously 1 mL of inoculums has brought back the concentration of plant extract medium to its half. So, the tube T_1 concentration moved from 100.00 mg/mL to 50 mg/mL. That of the tube T_2 from 50 mg/mL to 25 mg/mL till to tube T_9 with a real concentration of 0.19 mg/mL. That experience has been carried out in the same way for each tested extract. The nine (9) first tubes (from T_1 to T_9) are called « experimental tubes» and last tube (T_{10}) is called « reference tube or tube of growth». These full tubes have been incubated in an oven for 24 hours at 37°C. The experience has been reported three times [18].

The Minimum Inhibitory Concentration (MIC) corresponds to the first tube concentration where we can't observe any trouble visible to naked eye. From the MIC, the smallest concentration that allows at most 0.01 % of bacteria in the first suspension to survive within 24 hours corresponds to Minimum Bactericidal Concentration (MBC). It's determined by streaking on solid medium of 0.1 mL of the content of each tube that has a concentration superior or equal to the MIC. Therefore, the calculation of the ratio MBC/MIC of the extracts has permitted to determine their antibacterial power. According Berche *et al.*[22], when this ratio is greater than 4, the extract has bacteriostatic and bactericidal if this ratio is less than or equal to 4.

Phytochemical analysis

The phytochemical analysis of the different extracts of *P. laxiflora* have been based on the coloration and precipitation tests [23]. To better estimate the quantity of chemical constituents in the extracts, scores ranging from 0-3 (absent or present) have been allocated. Thus, the absence was symbolized by a score of 0, the presence in small quantities by a score of 1, the presence in average quantity score 2 and finally, the abundance by a score of 3.

ISSN: 0975-8585

Statistical analysis

Data were expressed presented and were presented as mean values ± SD (standard deviations). All the data were analyzed by one-way ANOVA and differences between the means were assessed with Dunnet/Turkey's multiple comparison tests. Differences were considered significant at $p < 0.05$. All analyses were carried out using Graph Pad software, version 5.01 (USA).

RESULTS AND DISCUSSION

Table 2 presents the different zones of inhibition of extracts of *P. laxiflora* tested in solid medium on two germs studied. All extracts have shown significantly ($p < 0.05$) antibacterial activity against the two bacteria tested. On the whole, the diameters of inhibitions have varied from 16 to 22 mm. The aqueous extract gave the zone of inhibition similar on whole of two strains tested (16-17 mm). As for the organic extracts (ethanol, methanol and ethyl acetate extracts), they have given on the two strains of the diameters of inhibition between 20 and 22 mm. These results show that the organic extracts inhibited significantly ($p < 0.05$) the growth of bacterial strains tested compared to aqueous extract. In addition, the witness control (T: DMSO/Distilled water) tested in the same conditions presented anydiameter of inhibition (0 mm) on these germs.

Table 2: Antimicrobial screening of extracts of *P. laxiflora* stem bark.

Bacterial strains	Zone of inhibition (mm)[a]				
	Etaq	$Eeth_{70\%}$	Emet	Eace	T
Shigella sp. 1177C10	17±0.026[a]	21±0.059[b]	21±0.028[b]	22±0.049[b]	0
S. aureus 204C12	16±0.054[a]	20±0.072[b]	20±0.014[b]	21±0.061[b]	0

a: at a concentration of 200 mg/mL; Values are mean± S.E.M. of 3 replications. Etaq : aqueous extract ; $Eeth_{70\%}$: ethanol extract ; Emet : methanol extract ; Eace : ethyl acetate extract, T : DMSO/distilled water in proportion 0.5: 0.5 ; V/V, Values with the same superscript character are not significantly different ($p < 0.05$).

Table 3: Antibacterial parameters compared of *P. laxiflora* stem bark extracts on bacterial strains

Extracts	Antibacterial parameters (mg/mL)	*S. aureus* 204C12	*Shigella* sp 1177C10
Etaq	MIC	100	100
	MBC	100	100
	MBC/MIC	1	1
	Power	Bactericidal	Bactericidal
$Eeth_{70\%}$	MIC	0.78	0.39
	MBC	3.12	0.78
	MBC/MIC	4	2
	Power	Bactericidal	Bactericidal
Emet	MIC	0.78	0.39
	MBC	1.56	0.78
	MBC/MIC	2	2
	Power	Bactericidal	Bactericidal
Eace	MIC	0.39	0.19
	MBC	1.56	0.39
	MBC/MIC	2	2
	Power	Bactericidal	Bactericidal

Mean values from three replicates are recorded; MIC : Minimum Inhibitory Concentration; MBC : Minimum Bactericidal Concentration; Etaq : aqueous extract ; $Eeth_{70\%}$: ethanol extract ; Emet : methanol extract ; Eace : ethyl acetate extract.

The extracts of *P. laxiflora* had strong bactericidal activity with MIC ranging 0.19 to 100 mg/mL and MBC ranging between 0.39 and 100 mg/mL. Aqueous extract (Etaq) gave a minimum bactericidal concentration of 100 mg/mL on the two germs tested. The organic extracts were more bactericidal against *S.*

aureus with one MBC of 1.56 mg/mL (ethyl acetate, and methanol extracts) and 3.12 mg/mL (ethanol extract) compared to the Etaq.The finding is similar with *Shigella sp*. For this strain the MBC obtained with the organic extracts are significantly ($p < 0.05$) better than aqueous extract. Against *Shigella sp*., the MBC was 0.39 mg/mL with ethyl acetate extract and 0.78 mg/mL for methanol and ethanol extracts. However, all extracts tested reveled bactericidal activities against study strains. Thus, the extracts which have induced the greatest inhibition diameters were more bactericides on the corresponding bacterial germs. This was the case of organic extracts compared to the aqueous extract. Ethyl acetate and methanol extracts have a same MBC (1.56 mg/mL) on *S. aureus.* By contrast, it is rather ethanol and methanol extracts who have also given a MBC identical to 0.78 mg/mL on *Shigella* sp. Among all extracts tested, ethyl acetate extract has been more bactericidal with a MBC of 1.56 mg/mL on *S. aureus* and 0.39 mg/mL for *Shigella* sp. (Table 3). This study further illustrates that pharmacological activity of a plant given may depend heavily on nature of the extraction solvent. This note has been already made by several authors, including the most recent was the work of Bouharb *et al.*[3].

In present study, bactericidal activity of ethyl acetate extract against multi-resistant strain of *S. aureus* was better (MBC = 1.56 mg/mL) compared to ethyl acetate extract of *Vernonia colorata* on a strain of *S. aureus* resistant to methicillin (Meti-R) presenting a MBC of 3.12 mg/mL [24]. Also, a study conducted by Ouattara *et al.*[25] demonstrated that ethyl acetate extract of *Vitex doniana* submitted an MBC of 3.12 mg/mL against *S. aureus* Meti-R. These results indicate that resistance of the same bacterial strain to plants extracts depends on nature of extract, but especially its bioactive compounds necessary to exert bactericidal action. In addition, the same strain of *Shigella* sp 1177C10 was more sensitive to ethanol extract of this study (MBC = 0.78 mg/mL) compared to those of *Clerodendrum splendens* which gave a MBC of 37.50 mg/mL [3]. This result could be explained by the fact that extracts of *P. laxiflora* tested would be richer in essential bioactive compounds. For comparison, aqueous extract gave an identical MBC on both bacterial strains. However, organic extracts were more bactericides on strain of Shigella sp. relative to *S. aureus*. Thus, reports $MBC_{S.\ aureus}/MBC_{Shigella\ sp}$ are between two (2) (methanol extract) and for (4) (ethanol and ethyl acetate extracts) (Figure 1).

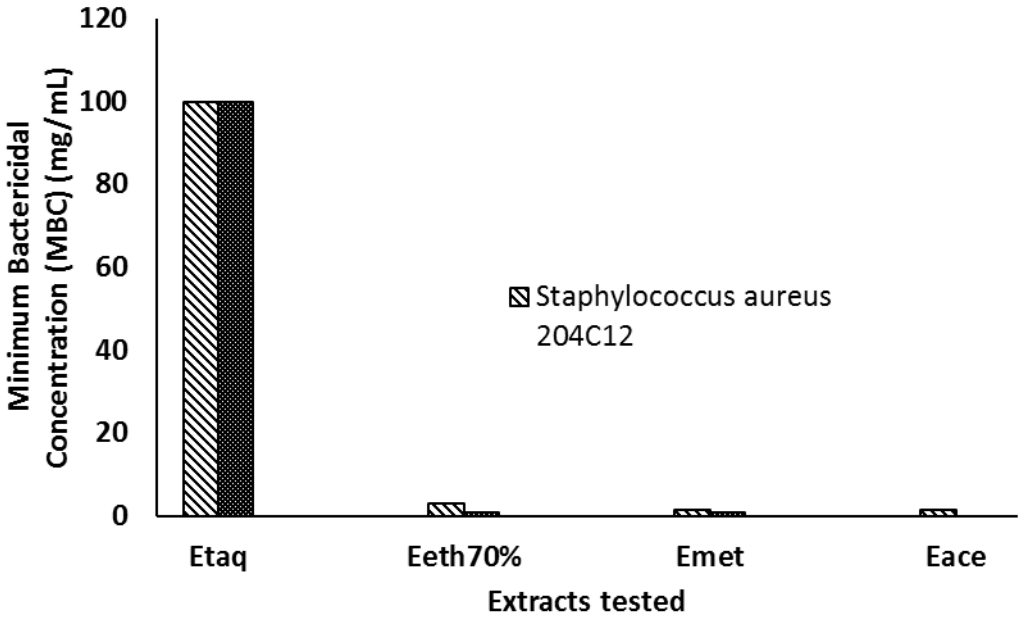

Figure 1: Comparison of Minimum Bactericidal Concentration (MBC) of extracts of *P. laxiflora* tested on germs studied

In addition to antibacterial tests, phytochemical analysis of different extracts *P.* laxiflora was conducted. Thus, the screening of extracts studied revealed the presence of phenolic compounds, flavonoids, tannins, cardiac glycosides, saponins, alkaloids, steroids and terpenoids. The composition of the bark of *P. laxiflora* shows thus a diversity in bioactive compounds. These results corroborate with those of other researchers [14,25]. Besides that, antimicrobial activity of most of these compounds has been already shown by several authors [26,27,28]. However, in case of present study, these bioactive compounds are differently presented in the extracts tested. The intensity of coloration observed in qualitative tests permit to conclude that there are more polyphenols, tannins and flavonoids in the organic extracts (Eeth, Emet and Eace) that

aqueous extract (Etaq) (Figure 2). Wealth of organic extracts in phenolic compounds compared to aqueous extract could explain their activities more interesting.

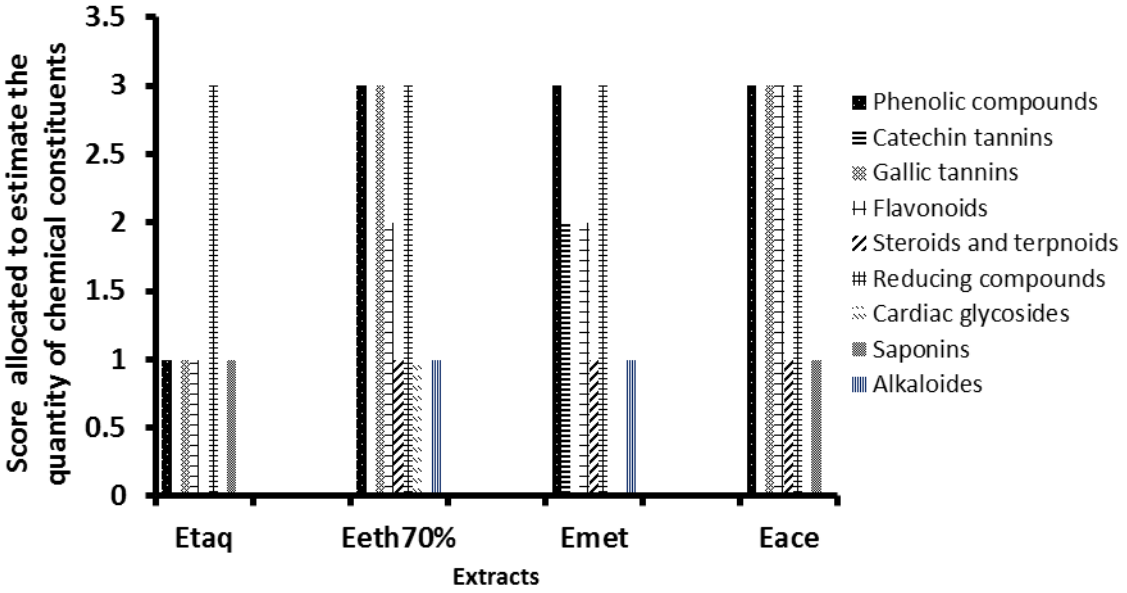

Figure 2: Phytochemical constituents of extracts of *P. laxiflora*

CONCLUSION

Antibacterial properties of *P. laxiflora* stem bark extracts have been demonstrated against *S. aureus* and *Shigella* sp., two multiresistant strains. According to results, ethanol, methanol and ethyl acetate are probably the best solvent for the extraction of bioactive compounds. However, all the extracts showed a significant antibacterial activity, included aqueous extract.

The results of this investigation give support to the traditional use of the stem bark of *P. laxiflora* in the treatment of infectious diseases caused by *S. aureus* and *Shigella* sp.

There is now scientific validation for the use of *P. laxiflora* for antibacterial activity as a medicinal plant. Further studies are ongoing in our laboratory to isolate, purify and characterize active principles in ethyl acetate extract because this plant can be a potential source of biologically important drug candidates.

ACKNOWLEDGEMENT

Our thanks go to Dr Koffi N'dri Emmanuel for his assistance during the writing of this publication.

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