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Research Article

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Phytochemical screening and evaluation of the antibacterial activity of bark extracts of *Pericopsis* (Afrormosia) *laxiflora* (Benth.) of *Escherichia coli* and *Klebsiella pneumoniae* ESBL

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ABSTRACT

The aim of our study was to evaluate the antibacterial properties of various extracts from the bark of Pericopsis laxiflora on Escherichia coli and Klebsiella pneumoniae, two strains of beta-lactamase producing extended spectrum (ESBL). The method of wells in the agar was used to test the sensitivity of bacterial strains while the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by the dilution method in liquid medium. The methanolic, acetatic and ethanolic extracts 70% gave inhibition zone diameters between 8 and 12 mm on the strains tested. Moreover, these extracts showed bactericidal powers of E. coli ESBL and K. pneumoniae ESBL with MIC and MBC ranging from 12.5 to 50 mg / mL. However, only the acetatic fraction gave the lowest CMB both E. coli ESBL (12.5 mg / mL) on K. pneumoniae ESBL (25mg/mL). The sensitivity of the bacteria tested justifies the use of this plant in traditional medicine to combat diseases in which the tested germs are involved including urinary tract infections and gastroenteritis.

Keywords: *Pericopsis laxiflora*, bactericidal, *Escherichia coli*, *Klebsiella pneumoniae*

INTRODUCTION

In recent decades, we see that the control of bacterial infections has become complex due to the emergence of bacteria resistant to many conventional antibiotics [1, 2, 3, 4, 5]. Deal with these health problems, the use of medicinal plants as potential sources of new active molecules finds its justification [6] and could provide a therapeutic response tailored to financial and socio-cultural environment populations [7]. Indeed, much research has proven that medicinal plants contain a variety of substances that several biological activities including antioxidant [8], anti-inflammatory and analgesic [9], antibacterial [10], antifungals [11] and even antiviral [12]. This was possible due to ethnobotanical studies conducted here and there on various floras [13].

Despite the significant progress recorded by different research teams, much remains to be done on medicinal plants, including their activities in connection with multiresistant bacteria. It is in this context that our choice is focused on *Pericopsis laxiflora* (Benth.) Van Meeuwen (Leguminosae) used in Côte d'Ivoire to the traditional treatment of many infections: headache, stomach ulcers, stomach aches, upset stomach, gastritis, enteritis, heart pain, abdominal pain [14]. It is also used throughout the dry forests and savannas of Africa. For example, in Guinea, it is used against shigellosis and colibacillosis [15]. In Ghana, this plant is used in the treatment of malaria [16] and as antiulcer ancestral area Benoue [17] in Nigeria.

The present study was undertaken to evaluate the activities of extracts *Pericopsis laxiflora* two Enterobacteriaceae (*Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL) responsible for several bacterial infections [18, 19, 20].

EXPERIMENTAL SECTION

Plant material

It consists of bark *Pericopsis laxiflora* (Benth.). These barks were collected in January 2010 in the north of Côte d'Ivoire in the village of precisely Lataha (Korhogo). Their authentication was performed by Professor Ake-Assi National Center Floristic (CNF) University Félix Houphouët-Boigny of Cocody-Abidjan where a sample is retained.

Bacterial strains

The bacterial carrier used in this study is composed of a strain of *Escherichia coli* (No 150C/12) and a strain of *Klebsiella pneumoniae* (No 141C/12), all both beta-lactamase producing extended spectrum (ESBL). They were provided by the Department of Bacteriology and Virology, Institut Pasteur de Côte d'Ivoire (IPCI).

Extract preparation

The *Pericopsis lax*iflora's bark harvested were washed, cut, dried in sunlight for two weeks and made into powder using a grinder type IKAMAG. These powders were used to prepare various extracts. Indeed, according to the methods described by Guede-Guina et al. [21], 100 g of plant powder were macerated in 1 L of distilled water and homogenized under magnetic stirring for 24 hours at 25°C using a magnetic stirrer RCT-type IKAMAG. The homogenate obtained was filtered successively twice cotton wool and once on Whatman paper No 2. The volume of the filtrate obtained is first reduced by means of a rotary evaporator Büchi type with the temperature of 60°C. Then the rest of the filtrate is evaporated using an oven type Med Center Venticell at 50°C to give a brown powder which is the total aqueous extract (Etaq). The same operation was performed using in place of distilled water a 70% ethanol or methanol or ethyl acetate to obtain respectively 70% ethanolic extract (Eeth70%), the methanol extract (Emet) or acetatic extract (Eace) [22]. All plant crude extracts thus formed are kept refrigerated until used for testing antibacterial.

Study of the antibacterial activity of different extracts

For each bacterial strain, inoculum was prepared by homogenizing 0.1 mL of a suspension opalescent 3 hours in 10 mL of Mueller-Hinton broth concentrate twice in order to obtain a bacterial load estimated at 5.10^6 CFU/mL. Also, a range of concentrations from 100 to 0.39 mg/mL was prepared by the method of double dilution [23] for each sample tested.

Determining zones of inhibition of growth

The method of the punch holes in the Mueller-Hinton agar was chosen instead of the method of discs loaded due to the limitations observed in the latter method for the non-dissemination of plant extracts. Thus, as in the conventional implementation of an antibiogram, each well of 6 mm in diameter was filled with 80 µL of extract concentration 200 mg/mL, taking care to separate two holes at least 20 mm. A control well was carried out for each bacterial strain with 80 µL of the solution mixture of DMSO/Sterile distilled water (V/V) [24, 25]. After a pre-release of 45 minutes at room temperature under the hood, the whole was incubated in an oven at 37°C for 18 to 24 hours. Meanwhile, the oxacillin (5 µg) and Cefoxitin (30 µg) were used as positive controls. After incubation, the action of the extracts is determined by measuring an area of growth inhibition (lack of colonies) around the well.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Dilution method in liquid medium was used to determine these antimicrobials parameters [23]. Thus, in a series of 10 hemolysis tubes numbered C_1 to C_{10} was introduced 1 mL of the bacterial inoculum. Then 1 mL of a plant extract well known concentration as the concentration range was added prepared in the same tubes. This distribution of plant extract is made so that 1 mL of plant extract 100 mg/mL is transferred to the tube C_1, the 50 mg/mL in the tube so C_2 to C_9 tube receive 1 mL of plant extract 0.39 mg/mL. The C_{10} has been tube, instead of plant extract, 1 mL of DMSO/distilled water (1/13, V/V) was used as control. This distribution of plant extract concentration is well known to each tube containing 1 mL of inoculum already reduced the concentration of the plant extract in the middle half. Tube and the concentration of C_1 increased from 100.00 mg/mL to 50 mg/mL, 50 mg/mL to 25 mg/mL for C_2 so on until a concentration of 0.19 mg / mL for T9. This experiment was performed identically for each extract tested. The nine (9) First tubes (C_1 to C_9) are called "experimental tubes" and the last tube (C_{10}) is rated "growth control tube or TC." These loaded tubes are incubated at 37°C for 24 hours. The experiment was done three times. The MIC is the concentration of the first tube where it finds no trouble visible to the naked eye. From the MIC, the lowest concentration that leaves no more than 0.01% survival of bacteria suspended starting 24 hours

corresponds to the CMB. It is determined by plating on solid medium 0.1 mL of each tube at a concentration greater than or equal to the MIC.

Antibacterial activity of the extracts tested

The antibacterial effect of different extracts tested was considered bactericidal or bacteriostatic depending on the MBC/MIC ratio. According Berche et al. [26], when this ratio is greater than 4, the extract has bacteriostatic and bactericidal if this ratio is less than or equal to 4.

Phytochemical screening

Phytochemical tests for tannins, flavonoids, alkaloids, sterols and polyterpenes, saponins, cardiac glycosides and reducing compounds were performed according to the methods described by Toure et al., Savithramma and al. and Shivakumar and al.[27, 28, 29].

RESULTS AND DISCUSSION

Table 1 shows the values of the diameters of the zones of inhibition of growth of bacteria tested. With the exception of the aqueous extract, there appears that each of the other three extracts has a well-defined activity on the growth of *E. coli* ESBL and *K. pneumoniae* ESBL.

Table 1: Diameters of inhibition of total extracts of stem bark of *Pericopsis laxiflora*.

	Diameter of the inhibition zones (mm)						
bacterial strains	Etaq	Eeth $_{70\%}$	Emet	Eace	Ox	FOX	C
E. coli BLSE	0	10	12	12	0	0	0
K. pneumoniae BlSE	0	8	9	10	0	0	0

Etaq : Aqueous total extract, Eeth$_{70\%}$: 70 % ethanolic Extract , Emet : methanolic extract, Eace : acetatic extract, C : Control (DMSO/Eau ; 0.5 : 0.5 ; V/V). Oxacillin (OX-5µg) and Cefoxitin (FOX-30µg)

The inhibition diameters are between 8 and 12 mm and are comparable to those obtained by other authors on clinical strains with total extracts of plants used in the treatment of various infections .[30].

According Biyiti et al. [31], a sample is judged active if it induces an inhibition zone of greater than or equal to 10 mm. Thus, against germs tested, the ethanolic extracts and methanolic were more active on *E. coli* ESBL with 10 and 12 mm zone of inhibition on *K. pneumoniae* ESBL (8 and 9 mm). As for the extract acétatique he has been active in both *E. coli* (12 mm) and *K. pneumoniae* (10 mm) compared to the aqueous extract and commercial antibiotics for which no zone of inhibition was observed (0 mm). Overall, the greatest sensitivity is found against the strain of E. coli whatever extract studied while the highest activity is observed with Eace whatever germ tested.
Parameter values antibacterial (MIC and MBC) as well as reports MBC/MIC determined are given in Table 2.

Table 2: Antibacterial parameters comparing total extracts of stem bark of *Pericopsis laxiflora* on the in vitro growth of the tested germs.

	antibacterial parameters (mg/mL)				
	Extracts	CMI	CMB	CMB/CMI	antibacterial effect
E. coli BLSE	Etaq	>100	>100	-	-
	Eeth $_{70\%}$	25	25	1	bactericidal
	Emet	12.5	12.5	1	bactericidal
	Eace	12.5	12.5	1	bactericidal
K. pneumoniae BLSE	Etaq	>100	>100	-	-
	Eeth $_{70\%}$	50	50	1	bactericidal
	Emet	25	50	2	bactericidal
	Eace	25	25	1	bactericidal

Etaq : Aqueous total extract, Eeth$_{70\%}$: 70 % ethanolic Extract , Emet : methanolic extract, Eace : acetatic extract

It follows from the analysis of these results that MIC values are consistent with those of the diameters of zones of growth inhibition. Indeed, extracts having a larger diameter induced inhibition showed smaller MIC values on the corresponding bacterial strains. This is the case of the Eace on strain of *E. coli* (MIC=12.5 mg/ml for 12 mm zone of inhibition) and on the strain of *K. pneumoniae* (MIC=25 mg/mL for 10 mm) or the Emet on *E. coli* (MIC=12.5 mg/mL for 12 mm). These results are comparable with those obtained by Traore et al. .[30] on strains of *Streptococcus pneumoniae* and *Candida albicans*. In addition, it should be noted that on *E. coli*, the inhibitory effects of Eace and Emet are equivalent (MIC=12.5 mg/mL for 12 mm). On other hand Eace is is two times more active than Emet against *K. pneumoniae* in so far efficiency report CMB_{Emet}/CMB_{Eace} is 2.

In addition, the MBC/MIC ratio was used to determine the bactericidal or bacteriostatic different extracts. According Berche et al. [26], when this ratio is less than or equal to 4, the extract has a bactericidal and bacteriostatic when this ratio is greater than 4. Thus, we can say that with the exception of the aqueous extract which power could be determined up to 100 mg/mL, the other three extracts exerted bactericidal effects against all bacterial strains tested.
Antibacterial activities observed are explained by the results of the phytochemical analysis of the extracts studied (Table 3) revealed the presence of compounds such as polyphenols, flavonoids, tannins, cardiac glycosides, alkaloids and sterols and polyterpenes.

Table 3: Chemical groups of total extracts of stem bark of *Pericopsis laxiflora*.

Extracts	Poly	Tan cat	Tan gal	Flav	Sterol et polyterp	Comp réd	Glyco card	sapo	Alkaloids	
									D	M
Etaq	+	-	+	+	-	+++	-	+	-	+
Eeth $_{70\%}$	+++	-	+++	++	+	+++	+	-	-	++
Emet	+++	++	-	++	+	+++	++	-	+	+++
Eace	+++	-	+++	+++	+	+++	+++	+	-	-

- : absence + : Présence ++ : forte présence +++ : très forte présence

Poly: polyphenol; Tan cat: catechic tannins; Tan gal: gallic tannins; Flav: flavonoids, sterol and polyterp: sterols and polyterpenes, Comp drafted: reducing compounds; Glyco card: cardiac glycosides; sapo: saponins ; D : Dragendorff's ; M :Mayer. Etaq : Etaq : Aqueous total extract, Eeth$_{70\%}$: 70 % ethanolic Extract , Emet : methanolic extract, Eace : acetatic extract

The antimicrobial activity of most of these compounds including flavonoids, tannins, alkaloids and terpenes has already been demonstrated by several researchers [32, 33, 34]. A high concentration of these compounds was detected in the methanolic and the acetatic extracts and justifying the more important activities of these extracts compared to the Etaq. From this result, we can deduce that unlike water, ethyl acetate and methanol are solvents that allow a better extraction of antimicrobial compounds virtues as those identified in the corresponding extracts. These results confirm those of Bssaibis et al. [25] who showed that the antimicrobial activities are related to the origin of the sample and the test strain as well as the nature of the solvent. This statement could also justify the fact that the aqueous extract had no effect on bacterial strains tested up to 100 mg/mL. For this concentration, the water could not really concentrate the active ingredients of the plant.

Moreover, it has been reported that *E. coli* is responsible for 40-70% of urinary tract infections in hospitals [18, 35, 36] and about 70% in the city [37] while hepatobiliary infections and neuro-meningeal postsurgical are caused by *K. pneumoniae* [38, 39]. The sensitivity of these strains to *Pericopsis laxiflora* extracts studied is of great importance because these strains are highly resistant to antibiotics used in clinical practice. Also, any antibacterial agent to which they are sensitive it deserves special attention. In addition, the concentrations at which these extracts remain active lead us to assert that this plant could be used against various diseases including gastroenteritis.

CONCLUSION

This work has allowed us to highlight the antibacterial properties of *Pericopsis laxiflora* on *E. coli* and *K. pneumoniae*, two bacteria that produce beta-lactamase and extended-spectrum involved in a large number of bacterial infections. The 70% ethanolic, methanolic and acetatic extracts showed bactericidal powers of *E. coli* ESBL E. and *K. pneumoniae* ESBL. However, acetatic extract was more active on *E. coli* ESBL and *K. pneumoniae* ESBL compared to other extracts tested.

In view of the results obtained in the present work, this plant could be used as phytomedicine to combat diseases in which the seeds are tested involved. To this end, it would be interesting to undertake studies of toxicity of the extracts which are found to be active and to consider the development of improved traditional medicines (ITM) after purification

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