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Anti-Gastroenteritis Activity of Methanolic Extract and Fractions of *Pericopsis Laxiflora* (Benth.)Stem Bark

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Abstract: *Over the last 20 years, the incidence of infectious diseases has increased as bacteria resistant to many conventional antibiotics have emerged. In this study, the attempt was made to evaluate the anti-gastroenteritis activity of various extracts of Pericopsis laxifloraagainst gram positive and gram negative bacteria. The metethanolic extract (Emet) was prepared by homogenization employing a maceration. Emet was redissout in distilled water and fractionated by using different organic solvents (n-hexane, dicholoromethane, ethyl acetate, and n-butanol). Ethyl acetate extract were fractionated on a column of silica gel. The antibacterial tests were performedby the methods of diffusion in solid and broth dilution coupled with the spread on agar plates.The acetal extract and the F_5 fraction (obtained after gel fractionation) were the most active. With the acetal extract, both strains of E. coli and K. pneumoniae ESBL recorded a MBC of 25 mg/mL. This extract also gave a MBC of 0.39 mg/mL on the three strains of S. aureus and Shigella sp.F_5 fraction gave a MBC of 12.5 mg/mL on E. coli strains against a MBC of 25 mg/mL for K. pneumoniae strain ESBL. Faced with this fraction F_5, all the S. aureus and Shigella sp. recorded a MBC of 0.19 mg/mL.The present study has shown that the extracts and fraction of P. laxiflora possess antibacterial activities against strains responsible for gastroenteritis.*

Keywords*: Pericopsis laxiflora, anti-gastroenteritis, methanolic extract, fractions*

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I. Introduction

Over the last 20 years, the incidence of infectious diseases has increased with the emergence of bacteria resistant to many conventional antibiotics(1). In developing countries, these infectious diseases account for about 90% of morbidity(2). This situation has worsened with the advent of HIV/AIDS, which causes various opportunistic infections due to immunodeficiency(3). These infectious diseases include gastroenteritis caused by various pathogenic microorganisms(4).

Despite the many existing antibiotics, gastroenteritis still has a high prevalence(5).Indeed, infectious gastroenteritis constitutes one of the main causes of morbidity and mortality among children under 5 years old. Each year, 1.3 billion of gastroenteritis episodes are observed in children in the world and four million die(5). This infection is especially problematic among infants, young children, and immune compromised persons.

In addition, the treatment of gastroenteritis remains difficult chemotherapeutically because of the existence of irrational or inappropriate polyprescriptions of antibiotics(6). Added to this is the resistance developed by the bacteria, a resistance due to the genetic instability of the strains as well as the limits of the antibiotic diagnostic tools. In other words, despite the existence of many antibiotics, the rate of therapeutic failures against gastroenteritis remains high(5). So it's a real public health problem.

In Côte d'Ivoire, as in other parts of the world, the epidemiology of gastroenteritis has always been a major concern for researchers because of the involvement of several micro-organisms(7). There are also socio-economic difficulties related to lack of hygiene(8-1). It should also be remembered that the majority of the population, especially African, can not afford effective drugs that are expensive for their purchasing power.

To this end, it would be desirable to find new molecules or seeded active molecules capable of responding to the needs of this situation (cost of production, primary and secondary resistance of the germs and

toxicity)[9-1].According to WHO statistics, almost 80% of the population in developing countries use medicinal plants and this has grown worldwide[10].

In this present research, the most important aim was to assess *in vitro* the antibacterial activity of methanolic extract (Emet) and various fractionsof *Pericopsis laxiflora*, a plant largely used in Cote Ivoire against bacteria responsible of gastroenteritis and food poisoning.

II. Material and Methods

Plant material

The barks of *Pericopsis laxiflora* were collected in the north of Côted'Ivoire in the village of Lataha (Korhogo). The plant was identified and authenticated by the Professor Ake-Assi, National Center Floristic (NCF) University Félix Houphouët-Boigny of Cocody-Abidjan where a sample is retained.

Preparation of extracts

The *P. laxiflora* s bark harvested were washed, cut, dried in sunlight for two weeks and made into powder using a grinder type IKAMAG.First, 100 g of this powder were macerated in 1 L of pur methanol 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 paper Whatman's N°2. The volume of the filtrate obtained isconcentrated to dryness under vacuum using a rotary evaporator (at 60°C) to remove methanol and give methanol extract (Emet)[1].

Second, 36 g of Emetwas redissout in water distilled and fractionated by using different organic solvents; n-hexane, dichloromethane, ethyl acetate, and n-butanol. Each fraction was filtered and then concentrated.The aqueous extract was so optneed[11].

Third, 10 g of ethyl acetate extract were fractionated on a column of silica gel eluting with hexane, ethyl acetate in the following proportions50/50 ; 33/67 ; 17/83 ; 8/92 ; 0/100 (v/v). Twelve[12] fractions (F_1 toF_{12}) were collected according to the chromatographic profiles. Both the extracts were collected in separate sterile vials and preserved at 4°C temperature to the antimicrobial tests**.**

Bacterial strains

The bacterial strains used for biological tests were provided by the Antibiotics Unit of NaturalSubstances and Survey of Resistance of Micro-organisms for Anti-infective (ASSURMI),Department of Bacteriologyat Pasteur Institute of Côte d'Ivoire (IPCI).

The strains used were:

Staphylococcus aureus sensitive to methicillin (*S. aureus* Meti -S);

Staphylococcus aureus resistant to methicillin (*S. aureus* Meti- R);

Escherichia coli ESBL (*E. coli* producing of β- lactamases at extended spectrum);

Klebsiella pneumoniae ESBL (*K. pneumoniae* producing of β- lactamases at extended spectrum),

Shigella sp resistant to Amoxicillin and Piperacillin (*Shigella sp*AMX-R & TZP-R).

Referenced strains of*E. coli* ATCC 25922 and*S. aureus* ATCC 25923 were also tested.

Antibiogram (agar diffusion method)

Antibacterial activity of different extracts of *P. laxflora*was performed with Mueller Hinton (Biorad, France)agar by well plate method[12]. Nutrient agar plates were seeded with overnight cultures of the test organisms.Wells, 6mm wide, were cut in the agar plates with cork borer and 80 µL of extracts at 200 mg/mL were pipettedand carefully added. A control well for each bacterial strain was added with 80 µL of mixture containingDMSO/sterile distilled water (V/V). The nutrient agar plates were incubated right side up at 37°C for 24 hours.

The zones of inhibition were then measured and the mean of two replicates recorded. Oxacillin (5 µg), and Cefoxitin (30µg) were used as standards antibiotics.

Determination of antibacterial parameters of different extracts

A concentration range of plant extract was prepared by the method of double dilution with concentrations ranging from 100.00 to 0.39 mg/mL for ethyl acetate extract and from 50.00 to 0.19 mg/mL for its fractions. The antimicrobial screening was performed using the method proposed by[12]. The tests were performed by introducing into a series of hemolysis tubes 1 mL of the solution of plant extract and 1 mL of bacterial inoculums. At the same time, in control tube, 1 mL of the solvent used to solve the extract (DMSO/distilled water to 1: 13, v/v) and 1 mL of bacterial inoculums were introduced. All the tubes were incubated at 37°C for 18 to 24 hours.

The results of antimicrobial screening were read looking through at daylight using human eye. The transparency of the tubes indicated the antimicrobial effect of the tested extract, while its turbidity shows its ineffectiveness (a sign of bacterial growth). The Minimum Inhibitory Concentration (MIC) will correspond to the concentration of the extract in the first tube with a clear content. The minimum bactericidal concentration (MBC) is the lowest concentration of extract that kills at least 99.99% of bacteria in culture. For its determination, the content of control tube was diluted to 10^{-4}, corresponding to 0.01% of survival bacteria in culture. The experimental tubes sowed antimicrobial effect from the MIC's one are transplanted by streaks of 5 cm on Mueller Hinton agar and incubated at 37°C for 24 hours. The first experimental tube in which the number of determined germs is less or equal to the dilution concentration (10^{-4}) corresponds to the MBC.

III. Results

The methanolic extract (Emet) was more active on the three strains of *S. aureus* and *Shigella* sp. with diameters of inhibitions which are arranged between 19 and 21 mm. For this same extract (Emet), the strains of *E. coli* and *K. pneumoniae* recorded inhibition diameters of between 9 and 12 mm.

Hexanic, dichloromethanic and butanolic extracts from the methanolic total extract (Emet) had no effect on *E. coli* and *K. pneumoniae* ESBL. However, these same extracts inhibited the growth of the three strains of *S. aureus* as well as that of *Shigella* sp. with inhibition diameters of 11 to 23 mm.

Aqueous extract had no effect on the germs tested (0 mm). The broadest spectrum of activity was observed with the ethyl acetate extract which inhibited the growth of all bacterial strains studied giving inhibition diameters between 10 and 23 mm (Table 1).

Table 1. Antibacterial efficacy of different extracts of *P. laxiflora*

Bacterial strains	Zone of inhibition (mm) at a concentration of 200 mg/mL								
	Extracts andstandard antibiotics								
	Emet	F_{hex}	F_{DCM}	F_{ace}	F_{but}	F_{aq}	Ox (5µg)	Fox (30µg)	T
E. coli ATCC 25922	9	0	0	10	0	0	0	0	0
E. coli BLSE	12	0	0	10	0	0	0	0	0
S. aureus ATCC 25923	19	11	13	22	15	0	25	25	0
S. aureus Méti-S	21	14	13	23	20	0	31	27	0
S. aureus Méti - R	20	12	12	21	14	0	0	0	0
Shigella sp.	21	12	13	23	18	0	0	0	0
K. pneumoniae BlSE	9	0	0	10	0	0	0	0	0

Emet : methanol extract ; Fhex : hexan extract ; F_{DCM} : Dichloro methan extract ; Face : ethyl acetate extract ;; Fbut : butanol xtract; Faq : aqueous extract; T : DMSO/distilled water in proportion 0.5: 0.5 ; V/V , Ox : Oxacillin, Fox : Cefoxitin

The new fractions obtained by column chromatography and tested against the different strains studied gave the results recorded in Table 2. With the exception of the fraction F_{12} (column residue) which showed a weak inhibition, all the other fractions (F_1 to F_{11}) significantly inhibited in vitro growth of the studied strains with larger diameters between 8 and 26 mm. The fraction F_5 proved to be the most active of all these new fractions thus giving higher diameters which vary from 12 to 26 mm.

Table 2. Antimicrobial screening of chromatography fractions of *P. laxiflora*

Bacterial strains	Zone of inhibition (mm)at a concentration of 200 mg/mL											
	Collected fractions by chromatography											
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}
E. coli ATCC 25922	11	10	11	11	**15**	10	11	10	11	11	9	7
E. coli ESBL	12	11	14	12	**15**	12	10	12	9	11	11	0
S. aureus ATCC 25923	22	22	21	20	**25**	19	21	22	18	20	17	9
S. aureus Meti-S	23	24	21	22	**26**	22	24	22	20	21	18	9
S. aureus Meti - R	21	20	19	20	**24**	18	21	22	16	18	15	8
Shigella sp.	22	23	22	21	**25**	20	22	21	21	22	20	10
K. pneumoniae ESBL	9	8	11	9	**12**	8	11	9	8	8	8	0

Table 3 shows the antibacterial parameters of the ethyl acetate extract and the fraction F_5. Thus, with the ethyl acetate extract, both strains of *E. coli* and *K. pneumoniae* ESBL recorded a MBC of 25 mg/mL. In addition, MBC of the three strains of *S. aureus* and *Shigella* sp was 0.39 mg/mL.
F_5 yielded a MBC of 12.5 mg/mL on *E. coli* strains against a 25 mg/mL for *K. pneumoniae* strain ESBL. All strains of *S.aureus* as well as *Shigella* sp. recorded a MBC of 0.19 mg/mL.

Table 3. Antibacterial parameters of ethyl acetate extract and F5 fraction of *P. laxiflora*

Bacterial strains	Antibacterial parameters					
	Ethyl acetate extract (Face)			F5 fraction (obtained by chromatography)		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
E. coli ATCC 25922	25	25	1	12.5	12.5	1
E.coli ESBL	25	25	1	12.5	12.5	1
S. aureus ATCC 25923	0.39	0.39	1	0.19	0.19	1
S. aureus Meti-S	0.39	0.39	1	0.19	0.19	1
S. aureus Meti-R	0.39	0.39	1	0.19	0.19	1
Shigella sp	0.39	0.39	1	0.19	0.19	1
K. pneumoniae ESBL	25	25	1	25	25	1

MIC:Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration

III. Discussion

The results of extracts from Emet would be justified by the principle of the series extraction which consists of using several solvents of different polarities in a precise order with a view to extracting all the extractable compounds solvent after solvent. Thus, during this serial extraction, as some bioactive compounds are concentrated others disappear.

Ethyl acetate extract (F_{ace}) activity was greater than that of the total extract (Emet). This detail could be explained by the purification and concentration of bioactive compounds. Indeed, the degreasing carried out by hexane followed by the action of dichloromethan would have extracted the molecules that can prevent any bactericidal action of the active ingredients. For the partitioned extracts that did not have bactericidal action on the tested strains, several reasons can be mentioned. These fractions do not contain the active principle (s) necessary for a bactericidal action but also the extracted molecules would be inhibited in their mode of action.Similar results were obtained during the fractionation of *Psidium guajava* extracts on strains of *S. aureus*, *S. faecalis*, *E. coli*, *S. typhi* and *K. pneumoniae*[13]. Different result obtained by other researchers in order to improve the pharmacological activities have also shown that the ethyl acetate used as extraction solvent concentrated at best the active ingredients of plants with bactericidal and fungicidal activities[14-12].

The interesting results of chromatography fractions of *P. laxiflora*could be explained by the fact that these different chromatographic extracts would be more rich in active compounds. Indeed, column chromatography with silica gel can in some cases extract, purify and concentrate the chemical groups.These results corroborate those of other authors. Indeed, Kuete *et al.*[15] also improved the antibacterial activities of the total *Citrus medica* juice extract (Rutaceae) using column chromatography with silica gel 60 (0.063-0.200 mm). Similarly, Golly *et al.*[12] used the same type of gel to improve the antibacterial activity of the acetal fraction of *Ceiba pentadra* against *S. aureus* and *P. aeruginosa*, two superinfection germs of Buruli ulcer.

For ethyl acetate extract (Face) and F5 fraction of *P. laxiflora*, the ratio MBC/MIC was 1 on all the germs studied. From this study, it is also noted that the three strains of *S. aureus* and *Shigella* sp. were the most sensitive, unlike the two strains of *E. coli* and *K. pneumoniae* that produce ESBL.

IV. Conclusion

The present study has shown that the extracts and fraction of *Pericopsis laxiflora* possess antibacterial activities against strains responsible for gastroenteritis.These results justify certain ethnopharmarcological uses of this plant. The observed antimicrobial potential of *P. laxiflora*fraction (F5) make it a candidate for bioassay guided and isolation of compounds which can possibly be developed into new lead structures for drug development programs against infectious diseases.

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