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Antibacterial Effect and Phytochemical Screening of the Aqueous Extract of the Stem Bark of *Piptadeniastrum africanum* Hook (Fabaceae) on the *in Vitro* Growth of the Enterobacteria Producing Beta-Lactamases with Broad Spectrum (EBLSE)

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KANGA Yao[1*], GUESSENND Nathalie[2], COULIBALY Kiyinlma[3], ZIRIHI Guédé Noel[1] et Mireille Dosso[2]

[1] *Botanical Laboratory, Unit of Formation and Biosciences Research, Félix Houphouët-Boigny University. PO Box, 582, Abidjan 22, Ivory Coast.*

[2] *Bactériology-Virology Laboratory, Unit of Antibiotics, the Natural Substances and the Monitoring of Resistances of the Micro-organisms to Anti-Infectious (ASSURMI), Pasteur Institute of Ivory Coast, PO Box, 490, Abidjan 01, Ivory Coast.*

[3] *Ufr biological Sciences, plant production department. Peleforo University Gon Coulibaly, PO Box, 1328 Korhogo, Ivory Coast*

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ABSTRACT

Objective: The objective of this study was to evaluate *in vitro* antimicrobial activity of the Total Aqueous Extract (TAE) of *Piptadeniastrum africanum* on the *in vitro* growth of enterobacterium but also to determine its phytochemical composition. Methodology and Results: The diffusion method by well in agar was used to determine antimicrobial activity. The results showed an activity of the total aqueous extract of *Piptadeniastrum africanum* on all the tested strains. However, the best activity was obtained on *Klebsiella pneumoniae* (18 mm). Also, the phytochemical screening of Total Aqueous Extract (TAE) showed the presence of several families of chemical compounds such as sterols/triterpenes, tannins, flavonoids, saponins, polyphenols and coumarins. Conclusion and application of the results: In the final analysis, the aqueous extract of *Piptadeniastrum africanum* is bactericidal on six enterobacterium but bacteriostatic on four strains of enterobacterium. From these analyses, the aqueous extract of the stem bark of this plant can be used as an antibacterial substance against bacterial infections.

INTRODUCTION

Enterobacteria are a very heterogeneous family in terms of their pathogenesis and ecology. The species which compose this family are indeed either parasitical, commensal, or even saprophytic. These bacteria are ranked first in the infection of GRAM negative bacteria, both in the community and in the hospital despite the appearance of antibiotics [1]. Indeed since their appearance, antibiotics have been the privilège way of fighting against bacterial infections. Among the many antibiotics, beta-lactam antibiotics are currently the most widely used in the treatment of bacterial infections all over the world. Thanks to their broad spectrum of action, innocuousness, efficacy and especially low cost [2]. However, due to their anarchic, inadequate and abusive uses in human health, we notice today a sudden appearance of multi-resistant bacteria [3]. In Côte d'Ivoire many cases of multi-resistance have been reported [4,5]. Today, the emergence and spread of multi-resistant bacteria in human populations have become a very serious public health problem [6]. The rapid progression of multi-resistance and the lack of real prospects for the discovery of new antibiotics led us to conduct an ethnobotanical survey on antimicrobial plants in the Haut-Sassandra region of Côte d'Ivoire. Following this investigation, *Piptadeniastrum africanum* Hook (Fabaceae) was selected for its antimicrobial properties and its frequency of use in traditional background. *Piptadenia africana* or *Piptadeniastrum africanum* is a large tree belonging to the family Fabaceae [7]. It is a leguminous plant of the subfamily of Mimosoideae whose crown is more or less tabular. It can reach a height from 50 to 65 meters. Dense foliage and dark green dominate the forest (Figure 1). It is supported on buttresses provided but sometimes very big. Its bipinnate leaves are composed of tiny leaflets suggestive of a fern [8].

The aim of this work is to evaluate the antibacterial properties of the aqueous extract of the stem bark of *Piptadeniastrum africanum* on the *in-vitro* growth of multi-resistant enterobacterium strains.

MATERIAL AND METHODS

Material

Plant material

The plant material consists mainly of powder from the stem bark of *Piptadeniastrum africanum* and identified at the National Floristic Center of Félix HOUPHOUËT-BOIGNY University of Côte d'Ivoire, Abidjan under number 21610 harvested on 21/05/1909 by herbarium.

Microbial material

The strains were supplied by the Antibiotics, Natural Substances and Microorganisms Surveillance to Anti-Infectives (ASSURMI) Unit of the Department of Bacteriology and Virology of the Pasteur Institute of Côte d'Ivoire (PICI). There are 9 clinical strains of enterobacteria and a reference strain of *E. coli* ATCC 25922 (Table 1).

Methods

Preparation of the extracts

The stem bark of *Piptadeniastrum africanum* harvested was rinsed with water and dried in the shelter of the sun. These dried plant organs were then reduced to a fine powder using an IKA-MAG RTC electric grinder. A gray powder is obtained. The aqueous total extract was prepared according to the method described by [9].

Total aqueous extract: One hundred grams (100 g) of bark powder are homogenized in 1 liter of distilled water in a Blender (Mixer) of Life's Superb brand (LS-317) for three times three minutes at ambient temperature. The homogenate obtained is filtered successively on hydrophilic cotton and then on Wattman paper (3 mm). With an oven set at 50 °C, the extraction solvent is eliminated. The dry evaporate is recovered in powder form which constitutes the total aqueous extract (TAE).

Evaluation of the antibacterial activity of the extracts

For the evaluation of the antibacterial activity of the plant extracts, two methods were used: the solid diffusion method and the liquid dilution method for the determination of MIC and MBC.

Sterility Test

This test made it possible to check the sterility of the extracts. 0.1 g (powder) extract was dissolved in 10 mL of thioglycolate culture medium. The whole was homogenized. After 24 hours of incubation at 37 °C, the culture medium is seeded on ordinary agar and Sabouraud in Petri dishes and then incubated for 24 hours at 37 °C. To ensure the absence of any fungal colonies, a reading was made every 24 hours over a total of ten days. The absence of any microbial colonies on the agar plates attests to the sterility of the extracts [10].

Preparation of the *inoculum*

The *inoculum* is an essential factor that can influence the results. It is, therefore, necessary that it be standardized. Two isolated colonies in a 24-hour culture, in a Petri dish, on a selective medium, were taken using a pear pipette equipped with a pear. The sample is used to make a suspension of optical density of 0.5 on the Mac Farland scale in 0.85 % NaCl. The suspension is diluted according to the multiplication speed of living germs. Thus, a dilution was carried out by adding a bacterial suspension of 100 µL in 10 mL of physiological water [11]. This new bacterial solution is the final *inoculum* with a concentration of 10^6 germs / mL.

Preparation of concentration range

It is prepared by the method of double dilution in liquid medium in a series of 7 labeled test tubes. For this purpose, 10 mL of sterile distilled water is put into the tube t_1 and 5 mL into all the other tubes. Then, a mass of 2 g of plant extract is dissolved in the tube t_1, then completely homogenized to give a concentration of 200 mg / mL. Half the volume of the tube t_1 (5 mL) is transferred into the tube t_2, later homogenized. This operation is repeated until the last tube, of which half of volume is rejected. The concentration range is then sterilized by filtration on a 0.45 µm membrane (millexgv) and stored in a refrigerator.

Efficiency test

It allows to eliminate extracts that show no antibacterial activity at the concentrations studied and retain only those that are active. It is carried out by the diffusion method in a solid medium. This consists in diffusing the extract or the antibiotic from a point of deposit in the agar. The efficiency of the extract is evaluated according to the diameter of the inhibition zone measured with a caliper [10]. The MH culture medium poured into the Petri dishes are swabbed with the prepared inoculum. The Petri dishes are left at room temperature for 15 min. 6 mm diameter wells are hollowed into the agar by pushing in the large end of a sterile Pasteur pipette and 50 µL of the extract is poured into these wells. A cup taken as a control receives 50 µL of distilled water. For the DMSO supplemented preparations, the control is prepared by adding 1 mL of DMSO to the 10 mL of distilled water. The dishes thus seeded are left at ambient temperature in the laboratory (26 °C.) for 15 minutes for pre-diffusion of the extracts. They are subsequently incubated for 24 hours after which the diameter of the inhibition zones is measured. The strain is resistant to the substance when the diameter measured is less than 8 mm, sensitive, when it is between 9 and 14 mm, very sensitive, when it is between 15 and 19 mm and extremely sensitive, when is greater than 20 mm [12].

Determination of antibacterial parameters

The antibacterial assays were carried out according to the liquid dilution method [13, 14] in a series of 7 experimental tubes, a growth control tube and a control tube for the test of sterility. 1 mL of the extract of the highest concentration is transferred into the T_1 tube, the next concentration in the T_2 tube, and so on until the lowest concentration in the T_7 tube. This approach resulted in ultimately reducing the concentration from T_1 to T_7, from double to single, i.e. from 100 mg / mL to 1.56 mg / mL. The sterility control tube receives 2 mL of Muller-Hinton culture medium. All of these tubes were incubated for 24 hours at 37 °C. This operation was repeated 3 times in succession. Thereafter, the contents of the tubes in which no disturbance was observed was used to inoculate the Muller-Hinton agar on 5 cm streaks starting with the first tube without turbidity and incubated at 37°C for 24 hours. Thus, the MIC was, therefore, the concentration of the first tube from which no disturbance to the naked eye was observed. After 24 hours of incubation at 37°C, the minimum bactericidal concentration (MBC) was determined by comparing the density of the streaks with that of the previously prepared A box.

Phytochemical characterization

The research of large chemical groups in total aqueous extract is carried out by a summary qualitative phytochemical analysis from the staining tests according to [15]. This analysis allowed to search compounds such as alkaloids, flavonoids, tannins, saponins, terpenes and sterols, coumarins and polyphenols.

RESULTS

Sterility Test

The sterility test of the total aqueous extract made it possible to check the sterility of the extract to be tested. The aqueous total extract of *Piptadeniastrum africanum* showed no signs of contamination after three readings separated with 24 hours of incubation.

Inhibition zones diameters

The diffusion method or cup method allowed to obtain the results reported in table 1 and 2. The aqueous extract of *Piptadeniastrum africanum* gave inhibition diameters ranging from 11±0.57 to 18±0.57 mm for hospital strains and a diameter of 13±57 for the reference strain of *Escherichia coli* ATCC 25922 at the concentration of 100 mg / mL. These results show a significant antibacterial activity of this aqueous extract of the stem bark of *Piptadeniastrum africanum* against the broad-spectrum beta-lactamase producing strains studied. The usual antibiotics have also given zones of inhibition. However, all tested strains were resistant to ceftazidime, amoxicillin and aztreonam according to the Antibiogram Committee of the French Society of Microbiology [11] (Table 2). The action of the stem bark maceration of *Piptadeniastrum africanum* at the concentration of 100 mg / mL against the strains is better than that of the antibiotics tested even if the charge of active principle of the crude aqueous extract is not known. At the concentration of 100 mg / mL of aqueous extract of the stem bark of *Piptadeniastrum africanum*, 50 % of the tested strains are sensitive and 40 % are very sensitive (Figure 2).

Antibacterial parameters (MIC and MBC)

The liquid dilution method used to determine Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) allowed to obtain results reported in table 3. The action of the macerated stem bark of *Piptadeniastrum africanum* is bactericidal on six

tested strains. The aqueous total extract showed MIC ranging from 6.25 mg/ml to 25 mg/ml. This extract is bactericidal on 50 % of the tested strains.

Phytochemical characterization

The result of the phytochemical screening is summarized in Table IV. Analysis of this result revealed the presence of secondary metabolites. Sterols and triterpenes, flavonoids, tannins, saponins, coumarins and polyphenols were determined in the aqueous extract except alkaloids.

Figure 1: *Piptadeniastrum africanum* (Forest of Bédiala Department of Daloa, Kanga 2016)

Table I: Codes and biological products of the studied strains

Codes	Strains	Organic products
531 UB/15	*Escherichia coli*	Urethral sampling
593 LC / 15	*Echerichia coli*	Urine
1091 C/15	*Salmonella sp*	Pus
590 LC/15	*Escherichia coli*	Pus
549 PP/15	*Escherichia coli*	Urine
421 YO/15	*Klebsiella pneumoniae*	Pus
563 UB/15	*Klebsiella pneumoniae*	Pus
792 YO/15	*Escherichia coli*	Urine
745 LC/15	*Escherichia coli*	Urine
ATCC 25922	*Echerichia coli*	Reference strain

Table II: Diameters of the inhibition zones (mm) of the total aqueous extracts of *Piptadeniastrum africanum* on 10 Enterobacterium

Bacterial strains		**Concentration in (mg/mL)**			**Control**	**antibiotics**	
Codes	Species	C_1 (100)	C_2 (50)	C_3 (25)	0 ± 0,00	AMC	FEP
531 UB/15	*E. coli*	11±0,57	6±0,00	6±0,00	0 ± 0,00	10± 0,00	9± 0,00
593 LC / 15	*E. coli*	6±0,00	6±0,00	6±0,00	0 ± 0,00	13± 0,00	8± 0,00
1091 C/15	*Sal sp*	14±0,57	6±0,00	6±0,00	0 ± 0,00	12± 0,00	11± 0,00
590 LC/15	*E. coli*	16±0,57	12±0,57	7±0,57	0 ± 0,00	6± 0,00	9± 0,00
549 PP/15	*E. coli*	15±0,57	12±0,57	10±0,57	0 ± 0,00	16± 0,00	6± 0,00
421 YO/15	*K. p*	18±0,57	16±0,57	14±0,57	0 ± 0,00	9± 0,00	6± 0,00
563 UB/15	*K. p*	17±0,57	15±0,57	13±0,57	0 ± 0,00	9± 0,00	6± 0,00
792 YO/15	*E. coli*	13±0,57	9±0,57	6±0,00	0 ± 0,00	14± 0,00	11± 0,00
745 LC/15	*E. coli*	14±0,57	13±0,57	12±0,57	0 ± 0,00	13± 0,00	14± 0,00
ATCC 25922	*E. coli*	13±0,57	10±0,57	9±0,57	0 ± 0,00	16± 0,00	14± 0,00

E. coli : ***Escherichia coli*** ; *Sal sp* : ***Salmonella sp*** *; K.p* : ***Klebsiella pneumonieae***

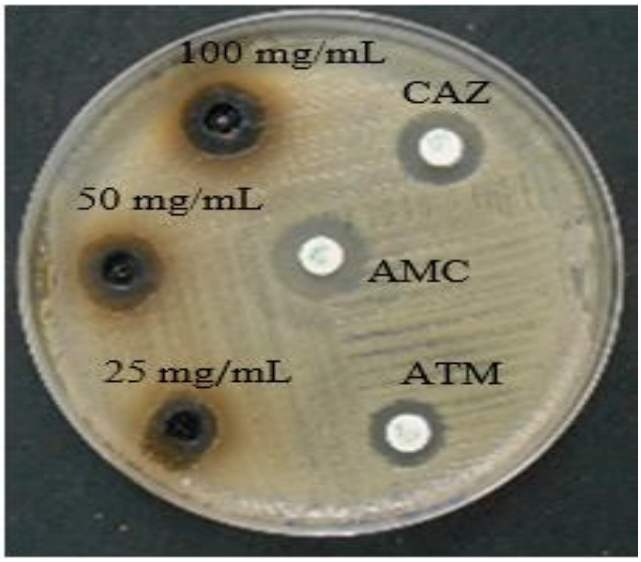

Figure 2: Inhibition diameters of TAE of *Piptadeniastrum africanum* and antibiotics on *Klebsiella pneumoniae* 563 UB/ 15

Table III: Antibacterial parameters of the total aqueous extract (TAE) of *Piptadeniastrum africanum* on the *in vitro* growth of 10 Enterobacterium

Codes	TAE			
	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Power
531 UB / 15	12,5	> 100	nd	bt
593 LC / 15	12,5	> 100	nd	bt
1091 C / 15	12,5	12,5	1	bc
590 LC / 15	6,25	12,5	2	bc
549 PP / 15	12,5	25	2	bc
421 YO / 15	12,5	50	4	bc
563 UB / 15	6,25	50	8	bt
792 YO / 15	12,5	100	8	bt
745 LC / 15	25	50	2	bc
ATCC 25922	12,5	12,5	1	bc

nd: not determined, bt: bacteriostatic, bc: bactericidal

Table IV: Chemical compound highlighted in the TAE of *Piptadeniastrum africanum*

Species	Extract	Chemical groups							
		Sap	Flav	Terp/ster	Tanins		Coum	Alc	Poly
					Gall	Cathé			
Piptadeniastrum africanum	TAE	+++	+	+	+	+	+	-	+

TAE: total aqueous extracts

+: presence of the chemical group

- : absence of the chemical group

+++: abundant presence of the chemical group

Sap : saponins ; **Flav** : flavonoid ; **Terp / Ster** : Terpenes / Sterols ; **Gall** : gallic ; **Cathé** : cathéchique ; **Coum** : coumarin ; **Alc** : alkaloids ; **Poly** : polyphénol

DISCUSSION

The aim of this study was to determine the antibacterial effect of the aqueous extract of the stem bark of *Piptadeniastrum africanum* on the enterobacterium producing broad spectrum beta-lactamases (EBLSE). To do this, only one type of extraction was carried out to obtain the aqueous extract. All tested EBLSE and ATCC strains were sensitive to the aqueous extract of the stem bark of *Piptadeniastrum africanum* compared to controls in a dose-response relationship except the *E. coli* 593 LC / 15 strain. This resulted in a gradual increase in the inhibition zone as the concentration of the aqueous extract increased (Tables II). For [16], an extract is considered active when it induces a zone of inhibition greater than or equal to 10 mm. The diameter of the inhibition zone being 90 % greater than 10 mm, we could say that the aqueous extract of the stem bark of *Piptadeniastrum africanum* is active. These results corroborate those of [8] and [17] who noted the antibacterial potency of *Piptadeniastrum africanum* on the *in vitro* growth of enterobacteria. Phytochemical study of the total aqueous extract *Piptadeniastrum africanum* stem bark showed the presence of the following chemical compounds (Polyphenols, Flavonoids, Coumarins, Saponins, Sterols / Triterpenes and Tannins). The presence of these chemical compounds in the organs of the plant is corroborated by studies by [8] which showed that the aqueous extract of *Piptadeniastrum africanum* could contain Polyphenols, Flavonoids, Coumarins, Saponins, Sterols / Triterpenes and Tannins. The presence of these chemical compounds could be at the origin of the antimicrobial activity of this plant because they are known for their antimicrobial properties [18, 19]. The obtained results confirm once again the efficiency of the extracts of the medicinal plants and their antiseptic power which comes to compete with that of the antibiotics. Numerous studies emphasize the antibacterial effect of natural active principle. Indeed, [20] report that the aqueous extract of *Marrubium vulgare* L leaves exerts strong inhibitory activity on strains of *Staphylococcus aureus* MTCC 740, *Staphylococcus epidermidis* MTCC 435 and an activity of lesser degree on *Proteus vulgaris* MTCC 426 and *E.coli* MTCC 443.

CONCLUSION

This work allowed us to demonstrate the antibacterial properties of the aqueous extract of the stem bark of *Piptadeniastrum africanum*. It demonstrates that this plant could be used to treat infectious diseases. However, this work must continue in order to isolate separately the phytomolecules responsible for the antibacterial activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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