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CYTOTOXICITY AND ANTIBACTERIAL ACTIVITY OF THE 70% ETHANOLIC EXTRACT OF THE STEM BARK OF *PIPTADENIASTRUM AFRICANUM* HOOK (FABACEAE).

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Abstract

In order to justify the traditional use of medicinal plants in the treatment of skin infections, Piptadeniastrum africanum Hook (Fabaceae), a plant used in traditional medicine in the Sassandra Region, in the treatment of bacterial diseases has been investigated. The 70% ethanolic extract of the stem bark was tested on six bacterial strains including three Staphylococcus aureus and three Pseudomonas aeruginosa as well as its cytotoxicity on HFF cells (Human Foreskin Fibroblasts). The dilution method in solid and liquid medium was used. The results show that the 70% ethanolic extract is active on all six bacterial strains with MIC ranging from 0.04 to 6.25 mg / mL. The 70% ethanolic extract showed a fungicidal action on all the strains studied and was not cytotoxic on the HFF cells studied. These results justify the traditional use of this plant against skin infections.

Keywords: *bacterial infection, ethanolic extract, Haut-Sassandra, Piptadeniastrum africanum,*

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INTRODUCTION:

In recent years, infectious diseases have been a major public health concern because of their frequency and severity [1]. The agents responsible for these various and varied diseases include fungi, bacteria, protozoa and viruses. To effectively fight these microbial infections, clinicians use antibiotics [2]. Unfortunately, the uncontrolled and often abusive use of antibiotics has ultimately resulted in increased resistance of the germs to these antibiotics rendering the treatments ineffective [3]. Faced with this situation, the search for new molecules has become a necessity. Faced with this reality of the inefficiency of synthetic molecules, the scientific community is moving towards natural substances, especially medicinal plants, in order to find new ones molecules that will not only contribute to the efficient fight against microbial diseases but also to promote traditional medicine. With this in mind, this study aims to evaluate the antibacterial activity of the stem bark of *Piptadeniastrum africanum* on the bacteria responsible for skin infections followed by a cytotoxicity test. *Piptadeniastrum africanum* is a plant of the family Fabaceae. It is widely used in the African pharmacopoeia to treat infections during circumcision, toothache and skin infections [4].

Material and methods

Plant material

It consists of the stem bark of *Piptadeniastrum africanum* Hook (Fabaceae), Figure 1.

A: Leafy twigs of *Piptadeniastrum* **B:** Foothills *Piptadeniastrum africanum africanum*

Figure 1: *Piptadeniastrum africanum*

Bacterial strains

The strains were provided by the Antibiotics, Natural Substances and Microorganisms Surveillance to Anti-Infectives Unit (ASSURMI) of the Department of Bacteriology and Virology of the Pasteur Institute of Ivory Coast (Table 1). These are two clinical strains of *Staphylococcus aureus*, and two clinical strains of *Pseudomonas aeruginosa* as well as two reference strains (*Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* 27853).

Table 1: List of strains studied

Bacteria	Codes	Origins	Phenotypes of resistance
S. aureus	ATCC 25923	-	Sensitive
S. aureus	505 PP/ 15	Pus	SARM ; RCFQ
S. aureus	310 CA/ 15	Urine	SARM ; RCFQ
P. aeruginosa	ATCC 27853	-	Sensitive
P. aeruginosa	255 C/12	Urine	PARC
P. aeruginosa	434 UB C/ 15	Aspiration Bronchitis	PARC

SARM: *Staphylococcus aureus resistant* to meticillin.
PARC: *Pseudomonas aeruginosa* resistant to carbapenems.
RCFQ: Cross-resistance to fluoroquinolones.
ATCC: American Type Culture Collection.

Cell lines

The cellular support consists of human HFF (Human Foreskin Fibroblasts) cells. These are human cells that testify to the toxic activity of an extract. They have the particularity of forming a cell layer after several days of culture (96 hours - 4 days), it is said that they are confluent, they stop dividing by contact inhibition. When these cells are in culture for only 24 hours, they are in a state of mitosis (or dividing cells). These HFF cells are cultured at 37° C. under 5% CO 2 in D10 medium (Gibco, supplemented with fetal calf serum 10%, glutamine 1%, penicillin 50 $U.ml^{-1}$ and streptomycin 50). ug / uL).

METHODS:

Collection of barks of plants

The bark of *Piptadeniastrum africanum* was harvested in August 2015 in the Sassandra Region (Ivory Coast). The samples of this plant have been identified at the National Center of Floristry of the UFR Biosciences of the University Felix Houphouet Boigny.

Preparation of plant extracts

After harvest, the barks were cleared of impurities, dried in the shade and in an air-conditioned laboratory for one week and then pulverized with an electric grinder. The fine powders obtained were stored in glass jars to prevent mold.

Total Aqueous Extract (ETA):

The dried barks of each plant were pulverized by a Moulinex type electric grinder. The powder obtained was used to prepare the various extracts. Thus ETA was obtained according to the following method [5-6]: One hundred grams of plant powder are extracted with distilled water by grinding in a mixer (Blender) three times three minutes at room temperature. The homogenate obtained is drained in a square of fabric and then filtered successively four times on hydrophilic cotton and then on Wattman paper (3 mm). The filtrate is evaporated at 50 ° C. using a venticell® type oven. The dry evaporate obtained constitutes the aqueous total extract (ETA).

Ethanolic extract 70% (EE70%)

The 70% ethanolic extract comes from the total aqueous extract, according to the following method [5]: Ten grams (10 g) of ETA are dissolved in 100 mL of an ethanol solution 70 % then homogenized in a Blender. After decantation in a separatory funnel, the supernatant is collected, filtered through cotton to remove any residue and dried in an oven (50 ° C). The powder obtained constitutes 70% ethanolic extract (EE70%).

Antibacterial study

Inoculum preparation for solid medium tests

The inoculum was prepared from two young 24-h colonies. They were emulsified in 2 ml of 85% NaCl suspension. Then, the optical density was adjusted to 0.5 Mac Farland using a densimate. A volume of 100 µl of this suspension was diluted in 10 ml of physiological saline (0.9% NaCl).

Sensitivity tests

These tests were performed using the solid medium diffusion method used by [7]. To do this, three solutions of concentration 100 mg / mL, 50 mg / mL and 25 mg / mL of extract were prepared. Petri dishes containing Muller-Hinton agar were inoculated by flooding with the prepared inoculum. Then, 6 mm diameter wells were dug by pressing the large end of a Pasteur pipette into the agar. These cups were then filled with the different concentrations of extracts. The assembly was incubated at 37 °C for 24 h. After this time, the inhibition diameter around each well was measured using a caliper. The sensitivity of the bacterial strains to extracts was assessed according to the criterion of [8]. Thus, a bacterium is said to be resistant if the inhibition diameter is less than or equal to 8 mm. Conversely, it is said sensitive if the diameter is between 9 and 14 mm and very sensitive when the diameter is between 15 and 19 mm and then extremely sensitive if the diameter is greater than or equal to 20 mm.

Preparation of the inoculum for liquid tests

Two 24-hour bacterial colonies were collected using a Pasteur pipette with a pear and emulsified in a test tube containing 10 mL of sterile Muller-Hinton broth. The mixture was incubated at 37 ° C for 3 h. After this incubation, 0.3 mL of this preculture was removed and diluted in 10 ml of sterile Muller-Hinton broth and then homogenized.

Preparation of the concentration range

A concentration solution at 100 mg / mL of the extracts selected for the determination of the antibacterial parameters was prepared. A series of dilutions was made from this solution to obtain ranges of concentrations ranging from 100 to 0.09 mg / mL.

Determination of antibacterial parameters

The determination of the antibacterial parameters was carried out by dilution in liquid medium according to the method used by [9]. Thus, in 10 experimental hemolysis tubes, 1 mL of each concentration range of plant extract was contacted with 1 ml of bacterial inoculum. The growth control tube received 1 ml of sterile distilled water in addition to the inoculum

while the sterility control received only sterile Muller-Hinton Broth (BMH). The tubes were incubated for 24 h at 37 ° C. After this incubation time, an observation with the naked eye was made and the lowest concentration for which no bacterial growth was observed corresponds to the Minimum Inhibitory Concentration (MIC). As for the Minimum Concentration Bactericidal (MBC), it refers to the concentration of a substance to obtain, after 24 hours of incubation at 37 ° C, 0.01% viable bacteria. His determination began with counting. This consisted of diluting the starting inoculum from 10^{-1} to 10^{-4} and sowing these different dilutions using a calibrated loop of 2 µL in streaks 5 cm long, on a Muller agar - Hinton (GMH) then incubate for 24 h. These Petri dishes were named A. After reading the MIC, the contents of tubes in which there was no visible growth was seeded on Muller-Hinton agar on 5 cm streaks. This series of petri dishes was named B. The MBC was determined by comparing the bacterial growth of the A and B boxes with the naked eye. Thus, the smallest concentration of the tube which has less than 0.01% of bacteria viable compared to the initial inoculum is the MBC. The MBC / MIC report clarified the antibacterial power of the extracts [10]. If the MBC / MIC ratio is less than or equal to four, the substance is said to be bactericidal. On the other hand, if it is greater than four, the substance is called bacteriostatic.

Cytotoxicity test:

The toxicity tests were carried out at LAPM (Laboratory Adaptation and Pathogenesis of Microorganisms) in Grenoble, France. To measure the toxicity of the ethanolic extract, the Human Foreskin Fibroblasts (HFF) cells were seeded in 96-well plates (CellStar) at 3000 to 5000 cells per well in 100 µl of D10 medium. These cells are kept in culture for 24 hours (dividing cells) or 96 hours (confluent cells). Subsequently they were exposed for 24 hours at different concentrations (0-1000 Yg / ml) plant extract solubilized in PBS buffer. This was done in triplicate. Viability was determined using 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide (MTT). The tetrazolium ring it contains is reduced in formazan by the mitochondrial succinate dehydrogenase of metabolically active cells, which precipitates and gives a violet color. The amount of precipitate formed is proportional to the number of living cells. In each well, MTT is added at a concentration of 500 µg / ml and incubated for 3h at 37 ° C. The formazan crystals are solubilized in 10 mM dimethylsulfoxide (DMSO). Measurement of optical density at 544 nm was made using a Safir spectrophotometer (Tecan); this measurement of absorbance will determine the relative amount of living and metabolically active cells [11]. Viability rate = (Abs544 nm extract / Abs544 nm control) × 100.

RESULTS:

Antibacterial activity

The 70% ethanolic extract was active on all the strains studied when referring to the diameters of inhibitions (figure 2). Inhibition diameters range from 23 to 25 mm for *Staphylococcus aureus* strains and from 10 to 14 mm for *Pseudomonas aeruginosa* strains (Table 2). The minimal inhibitory concentrations and the determined minimum bactericidal concentrations showed that the 70% ethanolic extract is bactericidal on all the strains with MIC between 0.09 and 12.5 mg / mL (Table 3) whereas the MBC range from 0.19 to 2 mg / mL (Table 3).

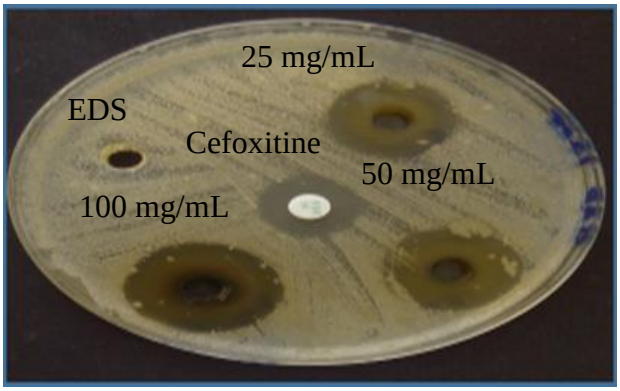

Figure 2: Inhibition Diameter of Ethanolic Extract 70% *Piptadeniastrum africanum* on *Staphylococcus aureus* 505 PP / 15

EDS: Sterile distilled water

Table 2: Diameters of the zones of inhibitions

Bacteria	Codes	Inhibition diameters in mm		
		C_1 :100 mg/mL	C_2 :50 mg/mL	C_3 :25 mg/mL
S. aureus	ATCC 25923	25 ±0,57	18±0,57	15±0,57
S. aureus	505 PP/ 15	23±0,57	21±0,57	15±0,57
S. aureus	310 CA/ 15	25±0,57	22±0,57	18±0,57
P. aeruginosa	ATCC 27853	14±0,57	12±0,57	11±0,57
P. aeruginosa	255 C/12	10±0,57	9±0,57	0
P. aeruginosa	434 UB C/ 15	10±0,57	9±0,57	0

S. aureus: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*

Table 3: MIC and MBC of 70% ethanol extract

Bacteria	Codes	70 % ethanol extract			
		MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	P
S. aureus	ATCC 25923	0, 09	0,19	2,11	bc
S. aureus	505 PP/ 15	0,39	0,19	0,48	bc
S. aureus	310 CA/ 15	0,09	0,19	2,11	bc
P. aeruginosa	ATCC 27853	12,5	25	2	bc
P. aeruginosa	255 C/12	12,5	25	2	bc
P. aeruginosa	434 UB C/ 15	6,25	12,5	2	bc

MIC: minimal inhibitory concentration; MBC: Minimal bactericidal concentration;
P: power; bc: bactericidal
S. aureus: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*

Cytotoxicity test of 70% ethanol extract of *Piptadeniastrum africanum*

The analysis of the results shows that the percentage of viability of human cells increases progressively as a function of the concentrations of plant extracts. Statistical analysis of variance shows that there is a highly significant difference ($p < 0.001$) between confluent cells and dividing cells. At 1000 µg / mL, the percentage of viability is 193% for confluent cells and 215% for dividing cells (figure. 3). This shows that *Piptadeniastrum africanum* is not toxic, but accelerates cell division (mitogenic effect).

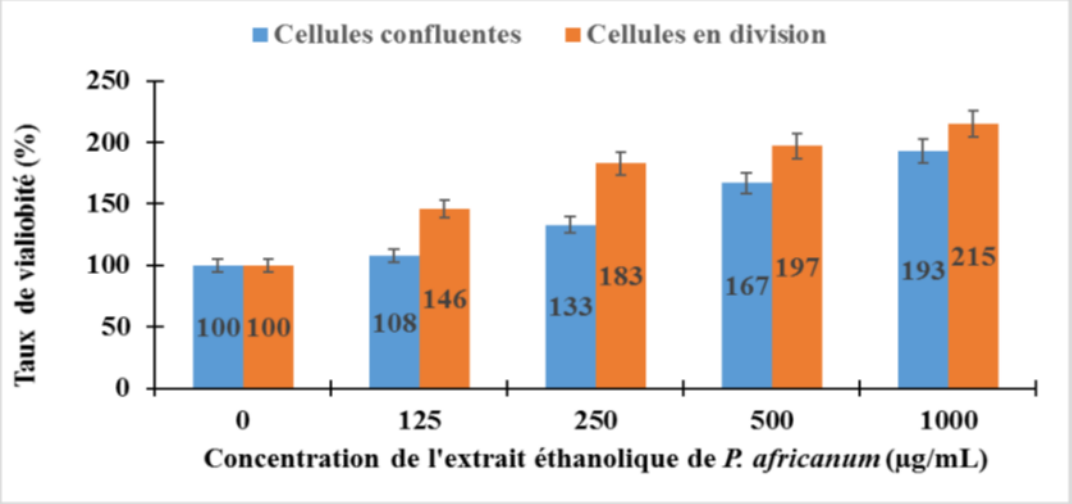

Figure 3: Viability of HFF cells in the presence of ethanolic extract of *P. africanum*
P. africanum: *Piptadeniastrum africanum*

DISCUSSION:

Efficacy results showed that all strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were susceptible to the 70% ethanolic extract of *Piptadeniastrum africanum*. All of these results obtained in solid medium shows that the activity of 70% ethanolic extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa* remains greater than that induced by synthetic antibiotics while these extracts are still raw products. The results of the efficacy tests also show that S. aureus are much more sensitive than *Pseudomonas aeruginosa*. This would be due to the different structures of the bacterial cell wall. Indeed, unlike Gram + bacteria, Gram-bacteria have an outer membrane in addition to the peptidoglycan [12]. The bacterial wall constitutes an exchange surface with the outside. The peptidoglycan is porous and passes many substances; this is not the case for the outer membrane of Gram-negative bacteria, which, in particular, oppose the penetration of hydrophobic antibiotics. As a result, the result is greater resistance of Gram-negative strains to the activity of plant extracts. The results obtained once again confirm the effectiveness of medicinal plant extracts and their antiseptic power that competes with that of antibiotics. Many works emphasize this antibacterial effect of natural active ingredients. Indeed, [13] reported that the aqueous extract of *Marrubium vulgare* L leaves exerted a strong inhibitory activity on *Staphylococcus aureus* strains MTCC 740, *Staphylococcus epidermidis* MTCC 435 and a lower degree activity on *Proteus vulgaris* MTCC 426 and *E. coli*. MTCC 443. These results corroborate the work of other researchers [4, 14] who noted the antibacterial potency of *Piptadeniastrum africanum* on Gram-positive and Gram-negative bacteria. Since the main target of these natural compounds is the bacterial membrane, the antibacterial activity of *Piptadeniastrum africanum* can also be explained by the presence of large groups of chemical molecules (flavonoids, alkaloids, saponins, terpenes and sterols, or even tannins).). In this study, ethanolic extracts of *Piptadeniastrum africanum* were more effective on all the bacteria studied. This could be justified by the fact that the hydroethanolic extracts concentrate better the active principles contained in the plant [5]. The MBC / MIC ratio revealed the bactericidal effect of 70% ethanolic extract on all six strains. Our results are in agreement with that of [4] who showed that *Piptadeniastrum africanum* had a bactericidal effect on S. aureus and P. aeruginosa. These results could justify the great solicitation of this plant in traditional environment in the treatment of cutaneous infections, abscesses and wounds. The result of the cytotoxicity test carried out on HFF cells showed that the ethanolic extract of *Piptadeniastrum africanum* is not toxic on human HFF cells. Indeed, according to [15], when the viability rate of an extract is strictly greater than 30% the extract is declared non-cytotoxic. But the ethanolic extract of *Piptadeniastrum africanum* showed a mitogenic effect on dividing HFF cells. This mitogenic effect is thought to be due to a chemical compound that acts as a growth factor favoring the multiplication of HFF cells. Our results are consistent with those of [16-17] who also showed that stem barks of *Bersama Abyssinica* (Fresen.) (Melianthaceae) and leaves of *Mallotus oppositifolius* (Geiseler) Müller. Arg (Euphorbiaceae) used in traditional medium in Ivory Coast had no cytotoxic effects on HFF cells.

CONCLUSION:

This study allowed us to show that 70% ethanolic extract of *Piptadeniastrum africanum* (Fabaceae) has antibacterial activity and is not cytotoxic on human cells (HFF). It is certainly for this reason that this plant is widely used in the Haut-Sassandra region in traditional environment against skin infections

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Conflict of interest

The authors declare no conflicts of interest

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