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BOTANICAL STUDY AND EVALUATION OF THE IN VITRO ANTIBACTERIAL ACTIVITY OF 70% ETHANOLIC EXTRACT OF PIPTADENIASTRUM AFRICANUM HOOK (FABACEAE) ON STAPHYLOCOCCUS AUREUS ISOLATED FROM URINE

Abstract: Objective: To know the antimicrobial medicinal plants of the Haut-Sassandra region (in the west center of Côte d'Ivoire) and to evaluate the antibacterial activity of the most requested species. Methods and results: An ethnobotanical survey conducted in the Haut-Sassandra Region using questionnaires allowed to gather information on the therapeutic uses of antimicrobial plants in the Region. It allowed to make a list of 58 species of plants. The taxon *Piptadeniastrum africanum* Hook (Fabaceae) is most in demand. The method of double dilution in the liquid and solid medium was used for the evaluation of the antibacterial activity of the extracts of the stem bark of this plant. The MBC / MIC ratio ranges from 1 to 2.25 mg / mL. The ethanolic extract (EE70 %) of the stem bark possesses bactericidal activity on all the tested strains of *Staphylococcus aureus*.

Conclusion: These results reveal that the Haut-Sassandra Region contains interesting antibacterial plants.

Key words: ethnobotany, antibacterial, MIC, MBC

Language: English

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INTRODUCTION

The resistance of microorganisms against classical antibiotics poses a real public health problem. Faced with this situation there is a pressing need for constant renewal of active principles [1]. The molecules sought must have various other chemical properties and mechanisms of action against these pathogenic microbes [2]. Medicinal plants represent a significant source of new medicines; especially since they have undesirable effect [3]. It therefore becomes logical to continue or even to intensify research in this direction knowing that plants remain an almost inexhaustible source of biomolecules. In addition, special attention is being paid to drugs and products of natural origin [4]. It is estimated that 80 % of the world's population depends mainly on traditional medicine for the treatment of diseases [5]. The dependence on remedies derived from indigenous plants is particularly marked in developing countries where Western medicine is often absent or simply too expensive. An ethnobotanical survey carried out in the Haut-Sassandra region of Cote d'Ivoire allowed us to select *Piptadeniastrum africanum* (Fabaceae), a plant used in the treatment of bacterial diseases in the traditional environment. The aim of this study is therefore to contribute to the valorisation of Côte d'Ivoire medicinal plants. It aims at evaluating the antibacterial activity of the ethanolic extract of the stem bark of *Piptadeniastrum africanum* on *Staphylococcus aureus*, germs that are often involved in urinary disorders.

1. MATERIAL

1.1. Vegetal material

The vegetal material used consists of the stem bark of *Piptadeniastrum africanum* harvested in the Haut-Sassandra region of Côte d'Ivoire in August 2015, the identification of which was made by the National Floristic Center of Côte d'Ivoire.

1.2. Bacterial material

The bacterial material consists of a reference and five multi-resistant strains of *Staphylococcus aureus* and it is provided by the Laboratory of Bacteriology and Virology of the Institut Pasteur of Côte d'Ivoire.

2. METHODS

2.1. Monographic study of *Piptadeniastrum africanum*

To allow easy identification of this plant in a natural environment, a full monographic study was carried out; it took into account:

- the botanical family of the plant ;
- general information on this botanical family ;
- detailed description of the plant ;
- some traditional therapeutic uses in the West African Pharmacopoeia.

2.2. Preparation of 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 extracts (total aqueous and ethanolic 70 %) were prepared according to the method described by [6].

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 room 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).

Ethanol / water partition: Ten grams (10 g) of the TAE are dissolved in 100 mL of a 70 % ethanol solution and then homogenized in a Blender [7]. After decantation in a separatory phial, the supernatant is collected, filtered on cotton to remove any residue and dried in an oven (50 °C.). The powder obtained constitutes the 70 % ethanolic extract (70 % EE).

2.3. Calculation of the yield

The yield is the amount of extract obtained from the vegetable powder. It is expressed as a percentage. In practice, it is determined by the ratio of the weight of the dry extract after evaporation to the weight of the powder of dry plant material used for extraction multiplied by 100. This results in the following formula: $Rd = (mx 100) / M$. (Rd: extraction yield in percentage, m: mass in gram of the dry extract, M: mass in gram of the powder of the drug).

2.4. Sterility test of 70 % ethanolic extract

The purpose of this test was to verify that the extract contained no bacteria or fungus. To do it, 0.1 g of this extract to be tested was placed in 10 ml of thioglycolate broth and incubated at 37 °C for 24 hours. After this time, the broth was seeded on a Petri dish containing the ordinary agar and another containing the Sabouraud agar and then incubated under the same conditions. The extract is declared sterile, if no colony is visible on the agar box [8].

2.5. Preparation of the bacterial inoculum

Two isolated colonies of 18 hours bacterial culture were homogenized in 10 mL of Muller-Hinton broth and incubated for 3 hours at 37 °C for pre-culture. A 0.1 mL sample of the pre-culture broth was diluted in a tube containing 10 mL of Muller-Hinton broth. This bacterial suspension produced was the bacterial inoculum of dilution 100. Two isolated colonies of an 18 hours bacterial culture were homogenized in 10 mL of Muller-Hinton broth and incubated for 3 hours at 37 °C for pre-culture. A 0.1 mL sample of the pre-culture broth was diluted in a tube containing 10 mL of Muller-Hinton broth.

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This produced bacterial suspension was the bacterial *inoculum* of dilution 100.

2.6. Numeration of bacterial *inoculum*

The bacterial *inoculum* was diluted 10 to 10-fold until 10⁻⁴ dilution. Four successive dilutions of 10⁻¹ to 10⁻⁴ are obtained. The initial bacterial inoculum and the four successive dilutions were inoculated with a 2 µL calibrated loop on Muller-Hinton agar plates with 5 cm long stripes constituting the A box.

2.7. Preparation of the concentration range of the substance

The concentration range was prepared in 16 test tubes numbered from T₁ to T₁₆ by the double-dilution method in liquid medium. This concentration range varies from 200 mg / mL to 0.006 mg / mL. For this purpose, 10 mL of sterile distilled water were put in the T₁ tube and 5 mL in all the other tubes. In the T₁ tube, 2 g of plant extract were dissolved at a concentration of 200 mg / mL. A 5 mL volume of the T₁ tube was transferred into the T₂ tube and then homogenized. This operation was repeated until tube T₁₆, 5 mL of tube T₁₆ was rejected. The contents of the tubes were filtered on a membrane (MILLEX GV®) of diameter 0.45 µm and stored in a refrigerator at 4 °C.

2.8. Antibacterial testing

The antibacterial tests were carried out using the dilution method into liquid medium [9,10] in a series of 16 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 to the T₁ tube, the next concentration in the T₂ tube, and so on until the lowest concentration in the T₁₆ tube. This procedure ultimately resulted in the concentration of T₁ to T₁₆ being reduced from double to single, ie from 100 mg / mL to 0.003 mg / mL. The sterility control tube receives 2 mL of Muller-Hinton broth. All of these tubes were incubated for 24 hours at 37 °C. This operation has been 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 striations 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.

3. RESULTS

3.1. Monographic study of the plant

Piptadeniastrum africanum belongs to the family Fabaceae

❖ Family of Fabaceae

Fabaceae are one of the largest families of flowering plants, with more than 730 genera and

19400 species distributed in both temperate and tropical environments [11]. They are herbaceous plants, shrubs, trees or lianas. The family is cosmopolitan of cold and tropical areas. The leaves are generally alternate, pinnate or trifoliolate and stipulated. However, there are some evolutions.

According to [12] the Fabaceae can be divided into 3 subfamilies:

- the subfamily of Mimosoideae ;
- the subfamily Caesalpinoideae ;
- the subfamily Papilionoideae or Faboideae.

❖ Some therapeutic uses of Fabaceae

Currently, several molecules are used in therapeutics and are extracted from various species of Faboideae: spartéine, a ganglioplégique alkaloid used in cardiology and obstetrics, isolated from *Cytisus scoparius* L. Link, rutin, a flavonoid used in phlebology, isolated from *Sophora japonica* L. or physostigmine from *Physostigma venenosum* Balf. The latter is a reversible inhibitor of cholinesterases, used as an antidote by parasympholytic intoxication and tested in the treatment of Alzheimer's disease [13].

❖ Description of the plant

Piptadenia africana or *Piptadeniastrum africanum* is a large tree. 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 [14].

❖ Therapeutic use of *Piptadeniastrum africanum*

It is used in the treatment of infections during circumcision. It is also used against childhood diseases; toothache and superinfections of the skin [14].

3.2. Extraction yield

We extracted 6.40 g of 70 % ethanol extract from 10 g of total aqueous extract, ie an extraction yield of the stem bark of *Piptadeniastrum africanum* of 64 %.

3.3. Sterility test

The sterility tests of the 70 % ethanol extract allowed to check the sterility of the extract to be tested. The 70 % ethanolic extract of *Piptadeniastrum africanum* showed no signs of contamination after three readings separated with 24 hours of incubation.

3.4. Antibacterial testing

After 24 hours of incubation at 37 °C., the increasing concentrations of the 70 % ethanol extract caused a gradual decrease in bacterial growth and a dose dependent on the turbidity of the culture medium and that for each bacterial strain studied (Figure 2). The values of the antibacterial parameters

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obtained for each bacterial strain are given in Table 1.



Figure 1 - Leafy twigs of *Piptadeniastrum africanum*

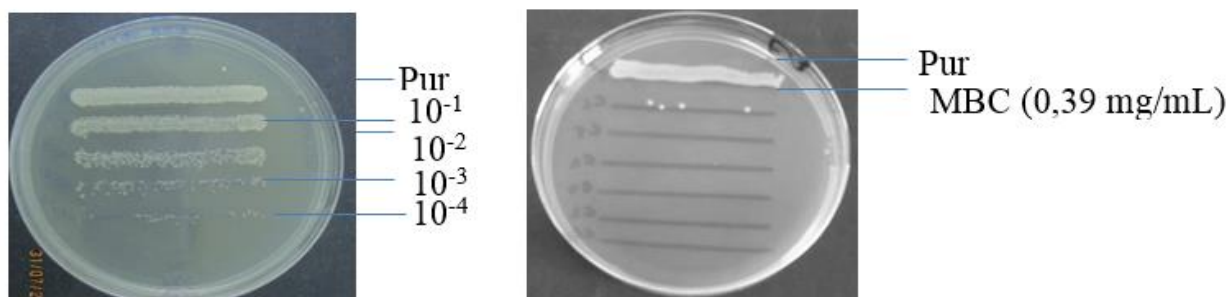


Figure 2 - MBC of the ethanolic extract of *Piptadeniastrum africanum* on *S. aureus* 1541C/14

Table 1
Values of the antibacterial parameters of the 70 % ethanolic extract of *Piptadeniastrum africanum* on the bacterial strains isolated from the urine

Bacterial strains	Code	MIC (mg/mL)	CMB (mg/mL)	MBC/MIC	Activity
<i>S. aureus</i>	ATCC	0,09	0,19	2,11	Bactericidal
<i>S. aureus</i>	408 C/14	0,04	0,09	2,25	Bactericidal
<i>S. aureus</i>	438 UB /15	0,09	0,09	1	Bactericidal
<i>S. aureus</i>	310 CA/15	0,09	0,19	2,11	Bactericidal
<i>S. aureus</i>	1541 C/14	0,39	0,78	2	Bactericidal
<i>S. aureus</i>	446 C/14	0,09	0,19	2,11	Bactericidal

MIC : Minimal Inhibitory Concentration ; MBC : Minimum Bactericidal Concentration

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4. DISCUSSION

The 70 % ethanolic extract gave a good extraction yield, ie 64 %. It could be a good extraction solvent for the active principle of the stem bark of *Piptadeniastrum africanum*. Tests of the 70 % ethanolic extract of the stem bark of *Piptadeniastrum africanum* in agar medium showed, as compared to the growth controls, a decreasing variation in the turbidity of the tubes as the concentration of the extract increases. This shows that this extract exhibits antibacterial activity by inhibiting the *in vitro* growth of strains of *Staphylococcus aureus* according to a dose-response relationship. All the tested bacterial strains showed different sensitivities to the 70 % ethanolic extract of the stem bark of *Piptadeniastrum africanum*. Thus, *S. aureus* 408 C / 14 is the most sensitive with a MIC of 0.04 mg / mL. Comparison of the activity of the 70 % ethanolic extract of the stem bark of *Piptadeniastrum africanum* on the basis of the MBC relative to the strains of infections and reference shows that the 70 % ethanolic extract is twice as active on *S aureus* 408C / 14 and *S. aureus* 412YO / 15 resistant to methicillin. We deduce that the 70 % ethanol extract exerts different inhibitory actions on the tested bacterial strains at different concentrations. Our results are in agreement with that of [14] which showed that *Piptadeniastrum africanum* had a bactericidal effect on *S. aureus*. The table of values of the antibacterial parameters also shows the different activities of the 70 % ethanolic extract of the stem bark of *Piptadeniastrum africanum* on the different strains of *Staphylococcus aureus*. The

activity ratios (MBC / MIC) are less than 4. According to [15], this fraction has a bactericidal activity with regard to the tested germs. Indeed, there are differences in the solubilizing capacity and the extraction of the solvents, with regard to the phytomolecules. According to [16], during the liquid-liquid extraction, the phytomolecules are distributed between the solvents according to their polarity and their solubility. We could say that the antibacterial substances contained in the stem bark of *Piptadeniastrum africanum* are more soluble in ethanol than in water. Ethanol would better then concentrate the active principle. The work of [17] have shown that the stem bark of *Piptadeniastrum africanum* contains, among others things, tannins, saponins which possess antibacterial properties. The presence of these chemical compounds could explain its activity on the strains of studied *Staphylococcus aureus*. All these results provide a scientific basis for the traditional use of this plant, especially in the treatment of wounds, suppurative collectors and urinary tract infections.

CONCLUSION

Our study showed that the 70 % ethanolic extract of the stem bark of *Piptadeniastrum africanum* has antibacterial activity on strains isolated from the urine. The extract of the stem bark of *Piptadeniastrum africanum* could therefore be a less costly alternative for the treatment of urinary tract infections.

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