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Botanical study and evaluation of the antifungal activity of *Piptadeniastrum africanum* Hook (Fabaceae) on the *in vitro* growth of *Trichophyton mentagrophytes*

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Abstract
Piptadeniastrum africanum Hook (Fabaceae) was selected in an ethnobotanical survey carried out in the Haut-Sassandra Region (Côte d'Ivoire) for its frequency of use against skin superinfections. The aqueous, ethanolic and residual extracts obtained from the powders of the bark allowed to evaluate the antifungal activity on a clinical strain of *Trichophyton mentagrophytes*. The three extracts (ethanolic, aqueous and residual) showed inhibitory activity with Minimum Fungicidal Concentration (MFC) between 0.195 mg / mL and 12.5 mg / mL.

Keywords: antifungal, aqueous extracts; ethanol extract, residual extract, Haut-Sassandra

1. Introduction
The populations of the Haut-Sassandra Region (Central-West) of Côte d'Ivoire live in the agricultural growing areas. Due to the remoteness of the health centers, they exploit medicinal plants extensively in the treatment of diseases that do not require an immediate evacuation in a hospital center [1]. Thus, the various extracts of *Piptadeniastrum africanum* are used for their antifungal properties in the treatment of skin and scalp diseases, mainly superficial mycoses. Superficial mycoses are microscopic fungal infections. Indeed, according to [2, 3] these molds are responsible for infections in the intertrigo spaces interdigito-plantar and onyxis of feet more frequent in warm climates. After an ethnobotanical survey carried out among the rural populations and poor social background of the Haut-Sassandra Region (Côte d'Ivoire), it appears that *Piptadeniastrum africanum* is much requested in the treatment of diseases of the skin and the scalp. The valorization of the medicinal plants of the Ivorian flora led us to evaluate *in vitro* the antifungal activity of the various extracts of this plant on *Trichophyton mentagrophytes* a mold involved in the superficial mycoses.

2. Materials and methods
2.1. Materials
- **Vegetal Material**

The vegetal material consists essentially 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**

Microbial material is composed of mold *Trichophyton mentagrophytes* from the mycology unit of the Training and Research Unit of the Faculty of Medical Sciences of Félix Houphouët-Boigny, University of Côte d'Ivoire.

2.2. Methods of study
2.2.1. Preparation of extracts
- **Total aqueous extracts (TAE)**

The extracts were prepared according to the method developed by [4]. This method can be summarized as follows: 100 grams of vegetable powder was extracted with one liter of distilled water by homogenization in a blender for 5 to 10 minutes. The homogenate obtained was drained in a square of white cloth and then filtered successively three times on hydrophilic cotton and once on 3 mm Wattman paper.

The filtrate obtained was dried in an oven at 50 °C and the powder thus obtained constituted the total aqueous extract denoted TAE.

- **Ethanolic extract (70 % EE)**

Five (5) grams of each aqueous total extract was dissolved in 10 mL of an aqueous-alcoholic solution containing 70 % ethanol and 30 % distilled water (V / V). After total depletion of the substance with the solvent in a mixer, an upper hydroalcoholic phase and a deposition were obtained using a separating phial [5]. The hydroalcoholic phase was collected, filtered on 3 mm Wattman filter paper and then dried in an oven at 50 °C. The powder obtained is the 70 % hydro-ethanolic extract, noted 70 % EE. The residual deposit was recovered and then dried in an oven at 50 °C to give the residual aqueous extract named RAE. The extracts obtained were stored in sterile glass jars. A total of six extracts were prepared.

2.2.2. 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 mass of the dry extract after evaporation to the mass of the powder of dry plant material used for extraction multiplied by 100. It is summarized according to the following formula:

r = (m x 100)/ M
r: extraction yield ;
m: mass in grams of the dry extract ;
M: mass in grams of the drug powder.

2.2.3. Evaluation of antifungal activity

2.2.3.1. Preparation of culture medium

Sabouraud agar was prepared by dissolving 42 g of agar powder in one liter of distilled water. This mixture was heated and stirred until complete homogenization was carried out on an IKAMAG-RTC magnetic stirrer. The mixture thus prepared was divided in a series of 12 tubes at a rate of 20 mL in the No. 1 tube and 10 mL in the other tubes (ranging from No. 2 to No. 12).

- **Incorporation of the extracts into the culture medium**

The incorporation of the plant extracts into the culture medium was carried out using the double dilution method in tilting tubes [5,6].Each series contains 10 test tubes containing the vegetable extract incorporated in the culture medium and two control tubes, one of which contains no plant extract for the control of the growth of the germs, the other without plant extract or germ for the control of the sterility of the medium culture. Test tubes contain concentrations ranging from 50 to 0.098 mg / mL. To carry out the double dilution, 1 g of plant extract was homogenized in tube 1 containing previously 20 mL of Sabouraud agar (to achieve the highest concentration of 50 mg / mL).

Then, half the volume of this homogeneous mixture was transferred to the following tube (n° 2), containing previously 10 mL of Sabouraud agar and homogenized. This operation was repeated successively for the other tubes to tube No. 10, to achieve the lowest concentration (0.098 mg / mL). For the latter tube, half the volume of the mixture was rejected. The 12 tubes prepared are sterilized in an autoclave at 121 °C for 15 minutes and inclined with a small base at laboratory temperature for the cooling and solidification of the agar [5, 7].

- **Préparation of the *inoculum***

The *inoculum* was prepared from young cultures of 5-10 days of *Trichophyton mentagrophytes*. This preparation was made by homogenizing one or two well-isolated colonies of taken fungal germs using a Koch loop in 10 ml of sterilized distilled water (each fungal species taken separately). This gives the mother suspension referred to as suspension 100 having a charge of 10^6 cells / mL. Suspension 10^{-1} was then prepared by diluting the mother suspension to the 10th, transferring 1 mL of the latter to 9 mL of sterile distilled water, thereby reducing the charge to 10^5 cells / mL. This last suspension will be used for antifungal tests [5, 7].

2.2.3.2. Antifungal tests in the presence of vegetable extract

The brothes previously prepared were seeded with 10 µl of the 10^{-1} suspension per tube (tube n°1 to n°11). This corresponds to 1000 seeded cells. For each of these tubes, the cultures were made in transverse striations until the depletion of 10 µL was achieved. After this step, all 12 tubes of each series were incubated in an oven at 30 °C for a period of 10 days for *Trichophyton mentagrophytes* [5, 7, 8]. The tests were repeated six times for each extract.

- **Colony count**

At the end of the incubation time, the colonies were counted by direct counting using a colony counter pen (Science Ware: Serial n°23283). Growth in the test tubes was expressed as a percent survival, calculated with respect to 100 % growth in the control tube of growth control [5, 7, 8].

The method of survival calculation can be summarized by the following formula:

$$S = \frac{n}{N} X\ 100$$

n = number of test tube colonies
N = number of control tube colonies
S = expressed survival as a %

- **Antifungal parameters sought**

The evaluation of the activity of the extracts is done by determining the values of the antifungal parameters (MIC, MFC, IC_{50}) and the appearance of the activity curves. The antifungal parameters can be defined as follows:

- MFC (Minimal Concentration Fungicide) is the lowest concentration of extract in the tube which gives 99.99 % inhibition compared to the control of the growth control or is the extract concentration of the tube which permit a survival 0.01 % relative to the control of the growth control [9];
- MIC (Minimal Inhibitory Concentration) is the lowest concentration of extract in the tube for which there is no visible growth to the naked eye [9];
- The IC_{50} (Concentration for 50 % inhibition) is the concentration which inhibits 50 % of the number of colonies relative to the growth control.

The IC_{50} is determined graphically from the antifogigram, which corresponds to the curve representing the evolution of the survival according to the concentration of vegetable extract [8, 10].

- **Détermination of fongicidia**

A subculture from the MIC tube is carried out on a new agar without plant extract. Thus, after three or ten days of incubation, the surface of the agar contained in the test tubes is slightly taken, seeded with a platinum loop on neutral agar and then incubated for 72 hours at room temperature [11].
Two cases may arise:

- if there are colonies, the extract is said to be fungistatic ;
- if there is no colonies, the extract is said to be fungicidal.

- **Criterion to compare the activities of the extracts**

➢ **The performances of the extracts**

The performances of the extracts are compared on the basis of several criteria (MFC, IC_{50} and the appearance of the activity curves). An extract is more active when these values of MFC and of IC_{50} are low. Thus an extract X_1 is considered more active than another extract X_2 if and only if the value of the MFC of X_1 is lower than that of X_2. But when two extracts X_1 and X_2 have the same value of MFC, then the most active extract is the one with the lowest IC_{50} value. As for the activity curve, its general appearance (decreasing, regular or irregular) and the relative value of its slope (strong, medium or low) indicates the potential of antifungal activity of the extract in question. The most active extract is the one whose activity curve has the strongest slope [3].

➢ **Activity reports**

The activity report determines how many times a given extract is more active than another. It is calculated by dividing the value of the highest MFC by the value of the lowest MFC. For example, if MFC (X_1) / MFC (X_2) = k, then the extract (X_2) with the lowest MFC value is k times more active than the extract (X_1) with the highest value of MFC.

3. Results

3.1. Botanical description of *Piptadeniastrum africanum*

According to [12], *Piptadenia africana* or *Piptadeniastrum africanum* Hook. (Fabaceae) called "Galuhi" in Bété (an ethnic group of Côte d'Ivoire) or "Bene Bene" in Gouro (an ethnic group of Côte d'Ivoire), is a large tree (Figure 1). It is a leguminous plant of the subfamily of mimosoideae whose crown is more or less tabular. It can reach height between 50 and 65 meters. The dense green foliage dominates the forest. It is supported on buttresses provided but sometimes very big. Its bipinnate leaves are composed of tiny leaflets that remind one of a fern. The ring border are indistinct or absent.

3.2. Therapeutic use of *Piptadeniastrum africanum*

It is used in the treatment of infections during circumcision. It is also used against childhood diseases; toothache and skin superinfections [13].

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

Fig 1: *Piptadeniastrum africanum*

3.3. Yields of extraction

The calculated yield with the aqueous extract of *Piptadeniastrum africanum* is 8 % per 100 grams of powders. As for the ethanol / water partition, the yield of *Piptadeniastrum africanum* showed the greatest yield with the ethanol extract (64 %) and the lowest yield with the residual extract (3 %).

3.4. Fungal tests

After 48 hours of incubation at 30 °C, a gradual decrease in the number of colonies as the concentration of the extracts increased in the test tubes was observed compared to the control. This decrease in the number of fungal colony is more remarkable for the 70 % ethanol fraction. This was also observed for both series (TAE and RAE). The experimental data revealed in the form of curves are shown in figure 2. The values of the IC_{50} (concentration for 50 % inhibition) and the MFC (minimum fungicidal concentration) are determined graphically. In general, the curves of the aqueous, ethanolic and residual extracts obtained have a progressively decreasing appearance, a steeper slope with the ethanolic extract. All of three curves intersect the x-axis.

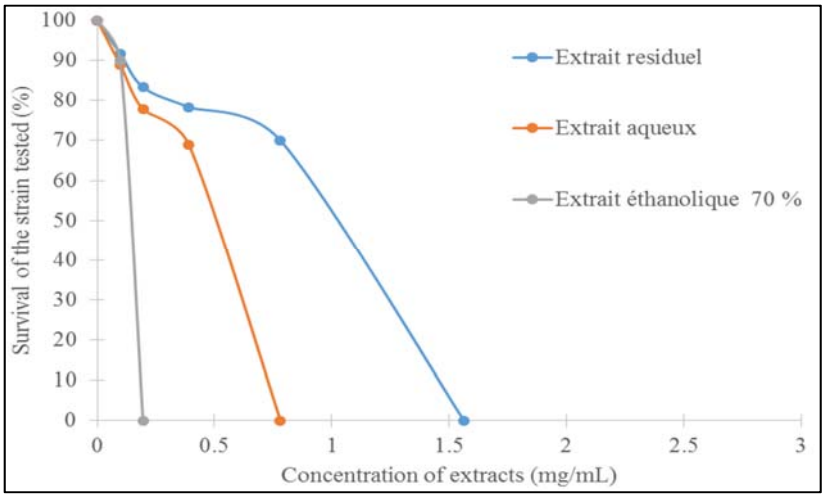

Fig 2: Sensitivity of *Trichophyton mentagrophytes* to extracts of *Piptadeniastrum africanum.*

Table 1: Values (mg / mL) of antifungal parameters of aqueous extracts, 70 % ethanolic and residual extracts of *Piptadeniastrum africanum.*

Extract of *Piptadeniastrum afrianum*	Antifungal parameters		
	MIC	MFC	CI_{50}
TAE	0,391	1,563	0,4
70% EE	0,195	0,195	0,2
RAE	1,563	12,5	1

Discussion

The different MFC of the three extracts (TAE, 70 % EE and RAE) observed suggest that *Piptadeniastrum africanum* has an interesting antifungal activity on *Trichophyton mentagrophytes*. With respective MFC values of 1.56 mg / mL, 0.195 mg / mL and 12.5 mg / mL, we can say that the ethanolic extract is eight times more active than the total aqueous extract and 64 times more active than the residual extract. This result is in agreement with that of [3]. This author has shown that for the extracts derived from the aqueous extract, the more the extractor solvent is rich in ethanol, the more active the extract prepared is, that is to say it concentrates a greater quantity of active principle from the aqueous extract. This result justifies the great use of the bark of this plant in traditional environment. This use is all the more justified because the *in vitro* tests give interesting results at low MFC (0.195 to 1.563 mg / mL). Several studies have been carried out with hydro-alcoholic vegetable extracts on the *in vitro* growth of *Trichophyton mentagrophytes*. A comparative analysis of the results obtained with those of [14] showed that the ethanolic extract of *Piptadeniastrum africanum* had a better activity than the hydroalcoholic extract of *Harrisonia abyssinica* (MFC = 50 mg / mL) but a lower activity than the ethanolic extract of *Mitracarpus villosus* (MFC = 3.125 mg / mL) [9]. Moreover, a comparative analysis of the results obtained with those of [15] also showed that on *Trichophyton mentagrophytes* the extracts of *Piptadeniastrum africanum* were 32 times more active than the ethanolic extract of *Eclipta prostrata* and eight times more active than the ethanol extract of *Acanthospermum hispidum* (MFC = 1.56 mg / mL). These results are justified by the work of [16], which showed that the effect of an extract is probably due to the synergy between the number of components, which when separated become inactive individually. Ethanol is therefore the solvent which better concentrates the active principles.

Conclusion

The various extracts of *Piptadeniastrum africanum* exerted an antifungal activity on the clinical strain of *Trichophyton mentagrophytes*. It presents therefore a practical interest in the struggle of superficial mycosis.

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