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Phytochemical Screening and Exploration of the Antifungal Activity of the Root Bark of *Piptadeniastrum africanum* Hook (Fabaceae) on *Trichophyton mentagrophytes*

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ABSTRACT

In Ivory Coast, the root bark of *Piptadeniastrum africanum* Hook (Fabaceae) is used in the Haut-Sassandra Region to treat a large number of infectious skin diseases of fungal origin. In order to scientifically justify the traditional use of this plant, we evaluated *in vitro* antifungal activity of the total aqueous extracts (TAE) and 70% ethanolic extract (70% EE) of the root bark of *Piptadeniastrum africanum* on *Trichophyton mentagrophytes*, a germ involved in various skin infections. The methodology followed is that of the *in vitro* culture of the germ on agar medium. Phytochemical screening has revealed a wealth of secondary metabolites such as saponosides, tannins, flavonoids, terpenes and sterols, polyphenols and coumarins. It is also apparent from this study that aqueous and hydroalcoholic extracts are fungicidal on *Trichophyton mentagrophytes*. The 70% ethanolic extract exercised better antifungal activity with a respective IC_{50} and MFC of 0.41mg/mL and 3.15mg/mL on *Trichophyton mentagrophytes*. Based on the results obtained *in vitro*, the 70% ethanolic extract of the root bark of *Piptadeniastrum africanum* could be a cheaper alternative against species of the genus *Trichophyton*.

INTRODUCTION

Superficial fungal infections are caused by microscopic fungi. They usually affect the skin, hair, nails and oral and genital mucosa. These are common infections, particularly in many African countries (Brindamour, 1995). At present, the treatment of superficial mycosis has a range of active chemicals on pathogenic fungi. However, the price of synthetic antifungal specialties remains relatively high. In spite of the available therapeutic arsenal, the majority of the population uses traditional medicine and pharmacopoeia. In the flora West African, there are several medicinal plants the virtues of which are often evoked, but active ingredients of which were not studied yet enough (Adjanohoun *et al*., 1979). It is the case of *Piptadeniastrum africanum* the medicinal plant which is used in western Africa in the treatment of the infections during the circumcision, the troubles of teeth and the dermatosis (Obame, 2009). In view of the interesting therapeutic properties of stem and root bark, *Piptadeniastrum africanum* is currently the subject of intense research activities (Onanga, 1997; Obame, 2009). In the Department of Issia (Ivory Coast) several health practitioners have revealed the use of the root bark of this plant in the treatment of athlete's feet and microsporic moths. On the basis of the information collected on this plant after an ethnobotanical survey, we undertook to study the antifungal activity of extracts of the root bark of *Piptadeniastrum africanum* on the *Trichophyton mentagrophytes* germ. This dermatophyte has a predilection for the keratin of the stratum corneum of the skin, hair, hair and nails in humans. It is responsible for superficial skin infections of the skin and integuments (Charlent, 2011; Rachid *et al*. 2014) Our study aims mainly to evaluate the antifungal activity of the two extracts of the root bark of *Piptadeniastrum africanum* on the in vitro growth of *Trichophyton mentagrophytes*, then to determine its chemical composition through a phytochemical screening.

MATERIAL AND METHODS

MATERIAL

Vegetal Material

The vegetal material consists essentially of powder from the root bark of *Piptadeniastrum africanum* and identified at the National Floristic Center of Félix Houphouët Boigny University of Ivory Coast, Abidjan under number 21610 harvested on 21/05/1909 by herbarium.

A: Leafy twig, B: Foothills, C: Root

Figure 1: *Piptadeniastrum africanum* Hook (Fabaceae)

Microbial material

Microbial material was 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 Ivory Coast.

METHODS OF STUDY

Preparation of extracts

Total aqueous extracts (TAE)

The extracts were prepared according to the method developed by (Zirihi *et al*., 2003). 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 Whatman 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 hydroalcoholic phase and a deposition were obtained using a separating phial (Zirihi *et al*., 2007). 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 % hydroethanolic extract, noted 70 % EE. The extracts obtained were stored in sterile glass jars. A total of six extracts were prepared.

Evaluation of antifungal activity

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 in to the culture medium was carried out using the double dilution method in tilting tubes (Guédé Guina *et al*., 1993; Zirihi *et al*., 2007). 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 plan 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 (no 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 (Zirihi *et al*., 2007, Bené *et al*., 2017).

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. This gives the mother suspension referred to as suspension 100 having a charge of 106 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 105 cells / mL. This last suspension will be used for antifungal tests (Bené *et al*., 2017).

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 no1 to no11). 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 (Kra, 2001; Bené *et al*., 2017). 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 no23283). Growth in the test tubes was expressed as a percent survival, calculated with respect to 100 % growth in the control tube of growth control (Kra, 2001). 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 (Ackah *et al*., 2008); MIC (Minimal Inhibitory Concentration) is the lowest concentration of extract in the tube for which there is no visible growth to the naked eye (Ackah *et al*., 2008);

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 (Kra *et al*., 2014).

Détermination of fungicidal activity

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 10 days at room temperature (Camara *et al*., 2016). 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 (Midgley, 1998).

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.

Phytochemical characterization

The research of large chemical groups in total aqueous extract and 70 % ethanolic extract are carried out by a summary qualitative phytochemical analysis from the staining tests according

to (Harborne, 1998). This analysis allowed to search compounds such as alkaloids, flavonoids, tannins, saponins, terpenes and sterols, coumarins and polyphenols.

RESULTS

Fungal tests

After 10 days 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). The experimental data revealed in the form of curves are shown in figure 2. The values of the IC_{50} (concentration for 50 % inhibition) are determined graphically. In general, the curves of the aqueous, ethanolic obtained have a progressively decreasing appearance, a steeper slope with the ethanolic extract. All of two curves intersect the x-axis. The different antifungal parameters have been determined in Table 1.

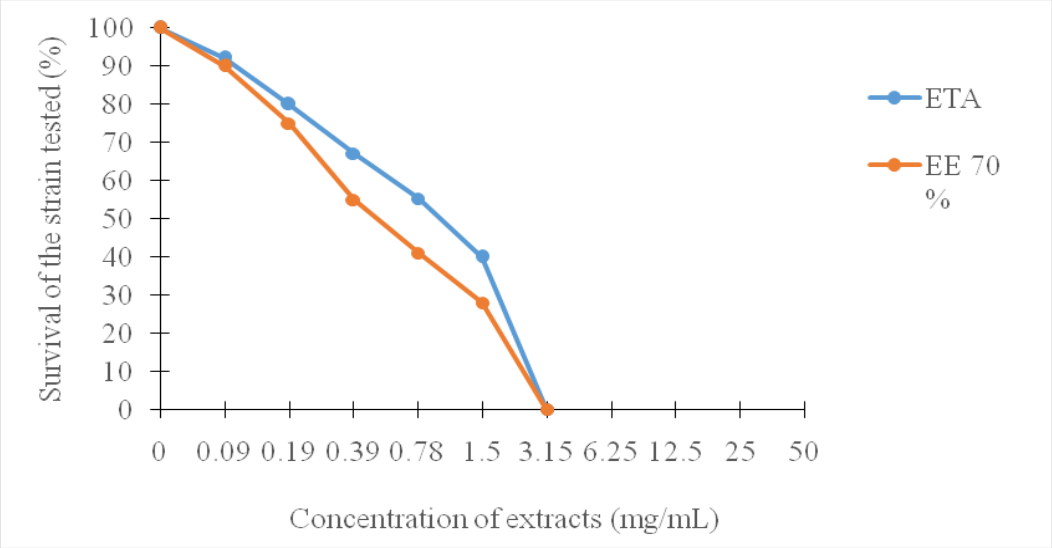

Figure 2: Sensitivity of *Trichophyton mentagrophytes* to extracts of *Piptadeniastrum africanum*

Table 1: Values (mg/mL) of antifungal parameters of aqueous extract and 70 % ethanolic of *Piptadeniastrum africanum* root bark

Piptadeniastrum afrianum root bark extract	Antifungal parameters		
	MIC	MFC	IC_{50}
Total aqueous extract	3,15	6,25	0,84
70 % ethanolic extract	3,15	3,15	0,41

Phytochemical characterization

Phytochemical screening revealed the presence of several groups of chemical molecules (Table II). There is an absence of coumarin in the two extracts, then an absence of terpene and sterol in the total aqueous extract and an absence of saponin in the 70% ethanolic extract.

Table II: Chemical compound highlighted in the TAE and 70 % EE of *Piptadeniastrum africanum* root bark

Species	Extract	Sap	Flav	Terp/Ster	Tanins		Coum	Alc	Poly
					Gall	Cath			
P. afrianum root bark extract	TAE	+++	+	-	+	+	-	++	+
	70% EE	-	+	+	++	+	-	+	+

P. africanum: *Piptadeniastrum africanum*

70 % EE : 70 % ethanolic extract

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

In order to verify the anti-infective virtues granted to the root bark of *Piptadeniastrum africanum*, we first prepared the aqueous extract since water is the most used solvent for the preparation of traditional recipes. On the microbial level, the analysis of our results shows that *Trichophyton mentagrophytes* is sensitive to both extracts of our study. Our results show that there is a gradual decrease in the number of colonies as the concentration of the extracts increases in the tubes. *Trichophyton mentagrophytes* is therefore sensitive to extracts in a dose-response relationship. After 10 days of incubation at 30°C, a clear and effective inhibition is observed in our series of tubes at different concentrations. The decrease of all the sensitivity curves illustrates that the extracts are active in a dose-response relationship. On the

basis of MFC or MIC, the determined efficiency ratio, namely MFC_{ETA} / $CMF_{EE70\%}$ is 1.98. This means that $EE_{70\%}$ is about 2 times more active on *Trichophyton mentagrophytes* than TAE. From this analysis, it can be deduced that $EE_{70\%}$ is more active than TAE. A difference in composition between the two extracts, related to the extraction method used according to Thangara *et al*. (2000), could explain these results. This observation is supported by several works including those of Moroh *et al*. (2008) and Bagré *et al*. (2011) who showed that ethanol allows a better concentration of active ingredients compared to TAE. According to these authors, when the total aqueous extract is changed to the ethanolic extract, certain chemical groups are eliminated and others are concentrated. Moreover, it is comparable to those found by Zihiri *et al*. (2003) who reported that the ethanolic extract of *Microglossa pyrifolia* is 100 times more active than its aqueous extract. In this study, the susceptibility of the germ tested to extracts of the root bark of *Piptadeniastrum africanum*, could justify the use of the root bark of this plant in the traditional treatment of microbial diseases such as dermatophytes in Ivory Coast. Phytochemical screening revealed the presence of tannins, flavonoids, alkaloids and polyphenols. The presence of these chemical compounds could be at the origin of the antifungal activity of the root bark of this plant because they are known for their antimicrobial properties (Akiyama *et al*., 2001; Min *et al*., 2008). A comparative analysis of our results on *Trichophyton mentagrophytes* with those of Bené *et al*. (2017) and Gbogbo *et al*. (2017) conducted previously on the same fungal strain showed that the 70% ethanolic extract of the root bark of *Piptadeniastrum africanum* is less active than *Bersama abyssinica* Fresen extracts. (Melianthaceae) (MFC = 0.195 mg / mL) and *Ficus platyphylla* del. (Moraceae) (MFC = 1mg / mL)

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.

Conflict of interest

We declare that we have no conflict of interest.

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