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PHYTOCHEMICAL STUDY AND *IN VITRO* EVALUATION OF THE ANTIFUNGAL ACTIVITY OF *HUNTERIA EBURNEA* PICHON (APOCYNACEAE) ON *TRICHOPHYTON MENTAGROPHYTES*.

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Abstract:

Objective: The objective of this study was to evaluate in vitro the antifungal activity of the total aqueous and ethanolic extract of Hunteria eburnea on the growth of Trichophyton mentagrophytes but also to determine its phytochemical composition. Methodology and Results: The method of incorporating plant extracts from the culture medium was carried out to determine antifungal activity. The results show a better antifungal activity of the 70% ethanolic extract (MCF = 3.12 mg / mL and IC $_{50}$ = 0.5 mg / mL) on Trichophyton mentagrophytes. Also, the screening of the aqueous total extract (ETA) and the ethanolic extract (EE) showed the presence of three families of chemical compounds such as saponins, alkaloids and sterols / triterpenes. Conclusion and application of the results: The ethanolic extract of Hunteria eburnea has a very good antifungal activity on Trichophyton mentagrophytes. As a result, the ethanolic extract of the stem bark of this plant can be used as an antifungal on Trichophyton mentagrophytes.

Key words: *Hunteria eburnea, aqueous extract, ethanolic extract, antifungal, screening.*

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INTRODUCTION :

The treatment of fungal infections remains difficult because of the limited number of effective principles and their very high cost and because of the emergence of strains resistant to certain common antibiotics [1-2]. Because of these problems there is a rapid spread of superficial mycotic infections and particularly in the elderly and immunocompromised [3-4]. In this context, researchers focused on the antifungal potential of various medicinal plants, since ancient times bioactive compounds contained in plants have been used to keep human being away from suffering [5]. An ethnobotanical survey was therefore conducted to find new and effective molecules that could be used as a template for the synthesis of other molecules [6]. Among the medicinal antimycotic selected, we have *Hunteria eburnea*, a plant highly sought by traditional health practitioners in the Region of Upper Sassandra (Ivory Coast), in the treatment of superficial mycosis. To appreciate the interest of *Hunteria eburnea* in human therapy, we proposed to evaluate the antifungal activity of the various extracts of this plant on *Trichophyton mentagrophytes*.

2. MATERIAL AND METHODS

2.1. Equipment

- **Plant material**

The plant material consists mainly of powder of the stem bark of *Hunteria eburnea* and identified at the National Center of Floristry Felix Houphouêt Boigny University of Ivory Coast, Abidjan under the number: 01/06/1981, Ake-Assi 15904 , Banco Forest (Figure 1).

A: bark of the trunk

1. B: leafy twigs

Figure 1: *Hunteria eburnea*

- Microbial material

Microbial material and *Trichophyton mentagrophytes* mold from the mycology unit of the Training and Research Unit of the Faculty of Medical Sciences of Felix Houphouêt Boigny University of Ivory coast.

2.2. Study methods

2.2.1. Preparation of extracts

- **Total aqueous extracts (ETA)**

The extracts were prepared according to the method developed by [`7]. This method can be summarized as follows: 100 grams of vegetable powder were 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 fabric and then filtered successively three times on hydrophilic cotton and once on wattman paper 3 mm. The filtrate obtained was dried in an oven at 50 ° C. and the powder thus obtained constitutes the total aqueous extract noted ETA.

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

Five (5) grams of each aqueous total extract was dissolved in 10 mL of a hydroalcoholic solution containing 70% ethanol and 30% distilled water (V / V). After total exhaustion of the substance with the solvent in a blender, an aqueous-alcoholic upper phase and a deposition were obtained through a separating funnel [8]. 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% aqueous ethanolic extract, noted as 70% EE. The extracts obtained were stored in sterile glass jars.

2.2.2. Assessment of antifungal activity

2.2.2.1. Preparation of the culture medium

Sabouraud agar was prepared by dissolving 42 g of agarose powder in one liter of distilled water. This mixture was heated and stirred until complete homogenization on a magnetic stirrer brand IKAMAG-RTC. The medium thus prepared was distributed in series of 12 tubes at a rate of 20 ml in tube No. 1 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 according to the method of double dilution in bent tubes [9]. Each series consist of 10 test tubes containing the plant extract incorporated in the culture medium and two control tubes, one without a plant extract for the control of growth of the germs, the other without a plant extract or germ for the control of the sterility of the growth medium culture. The test tubes contain concentrations ranging from 50 to 0.098 mg / mL. To perform the double dilution, 1 g of plant extract was

homogenized in tube No. 1 previously containing 20 ml of Sabouraud agar (to achieve the highest concentration 50 mg / ml). Then, half the volume of this homogeneous mixture was transferred to the next tube (No. 2), previously containing 10 mL of Sabouraud agar and homogenized. This operation was repeated successively for the other tubes up to tube No. 10, to achieve the lowest concentration (0.098 mg / ml). For this last tube, half the volume of the mixture was rejected. The 12 prepared tubes are autoclaved at 121 ° C for 15 minutes and sloped with small pellet at laboratory temperature for cooling and solidification of the agar [10]. .

2.2.2.2. Preparation of the inoculum

The inoculum was prepared from 5-10 day old cultures of *Trichophyton mentagrophytes*. This preparation was made by homogenizing one to two well isolated colonies of fungal germs collected, using a loop of Koch, in 10 mL of sterilized distilled water (each fungal species taken separately). This gives the so-called suspension suspension 100 mother having a charge of 106 cells / mL. The 10^{-1} suspension was then prepared by diluting the mother suspension to 10 th, transferring 1 mL of this suspension into 9 mL of sterile distilled water, thereby reducing the load to 105 cells / mL. This last suspension will be used for antifungal tests [10].

2.2.2.3. Antifungal tests in the presence of the plant extract

The culture media previously prepared were inoculated with 10 µl of the suspension 10^{-1} per tube (tube No. 1 to No. 11). This corresponds to 1000 seeded cells. For each of these tubes, the cultures were made in transverse streaks until the 10 µL was exhausted. 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* [10-11]. The tests were repeated six times for each extract.

- **Enumeration of colonies**

At the end of the incubation time, colonies were counted by direct counting using a colony counter pen (Science Ware: Serial No. 23283). Growth in the test tubes was expressed as percent survival, calculated as 100% growth in the growth control control tube [10-11]. The method of calculating the survival can be summarized by the following formula n

S= n/N X 100
n = number of test tube colonies
N = number of colonies of the control tube
S = survival expressed in %

- **Desired antifungal parameters**

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

- CMF (Minimal Fungicidal Concentration) is the smallest concentration of extract in the tube that gives 99.99% inhibition compared to the control of growth control or it is the concentration of extract of the tube that leaves a survival 0.01% relative to the growth control control [12];
- MIC (Minimal Inhibitory Concentration) is the smallest concentration of extract in the tube for which there is no visible growth with the naked eye [12]

the IC50 (concentration for 50% inhibition) is the concentration which inhibits 50% of the number of colonies relative to the growth control.

The IC50 is determined graphically from the antifungal diagram, which corresponds to the curve representing the evolution of the survival as a function of the concentration of plant extract [11-13].

- **Determination of fungicidia**

A subculture from the MIC tube is made on fresh agar without plant extract. Thus, after three or ten days of incubation, the surface of the agar contained in the test tubes is slightly removed, seeded with a platinum loop on neutral agar and incubated for 72 hours at room temperature. Coulibaly, [14].

. Two cases may occur:

- if there are colonies, the extract is said to be fungistatic;
- if there is absence of colonies, the extract is called fungicide.

- **Criterion for comparing the activities of extracts**

➢ The performances of the extracts

The performances of the extracts are compared on the basis of several criteria. The values of the antifungal parameters (MCF, IC_{50} and the shape of the activity curves). An extract is all the more active as these values of MCF and 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 MCF of X_1 is lower than that of X_2. But when two extracts X_1 and X_2 have the same value of MCF, 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 (high, medium or low) provides information on the potential for antifungal activity of the extract in question. The most active extract is the one whose activity curve has the steepest slope [7]

➢ 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 MCF by the value of the lowest MCF. For example, if MCF (X_1) / MCF (X_2) = k, then this means that the

extract (X_2) with the lowest value of MCF is k times more active than the extract (X_1) with the highest value of MCF.

2.2.3. Phytochemical Screening

The detection of phytochemical groups such as alkaloids, flavonoids, tannins and saponosides was performed by qualitative staining methods according to [15].

3. Results

3. 1.Antifungal test

After 72 hours of incubation at 30 ° C., a progressive decrease in the number of *Trichophyton mentagrophyte*s colonies compared to the control is observed as the concentrations of the plant extracts increase in the experimental tubes. This is observed for all series of the two extracts (Figure 2 and Figure 3). Net and effective inhibitions were obtained at different concentrations according to the extracts. The values of MCF (minimum fungicidal concentration) for the two extracts are recorded in Table I. The experimental data translated in the form of sensitivity curves are summarized in Figure 3. In general, the curve of the ethanolic extract has a declines with a steeper slope than the aqueous extract (Figure 4).

3.2. Phytochemical Screening

Table II gives the results obtained during the phytochemical screening of *Hunteria eburnea*. The tests carried out reveal the presence of various secondary metabolites in the extracts evaluated. Saponins were the only chemical compounds present in the aqueous total extract, whereas in the 70% ethanol extract, the presence of sterols / triterpenes and alkaloid was noted.

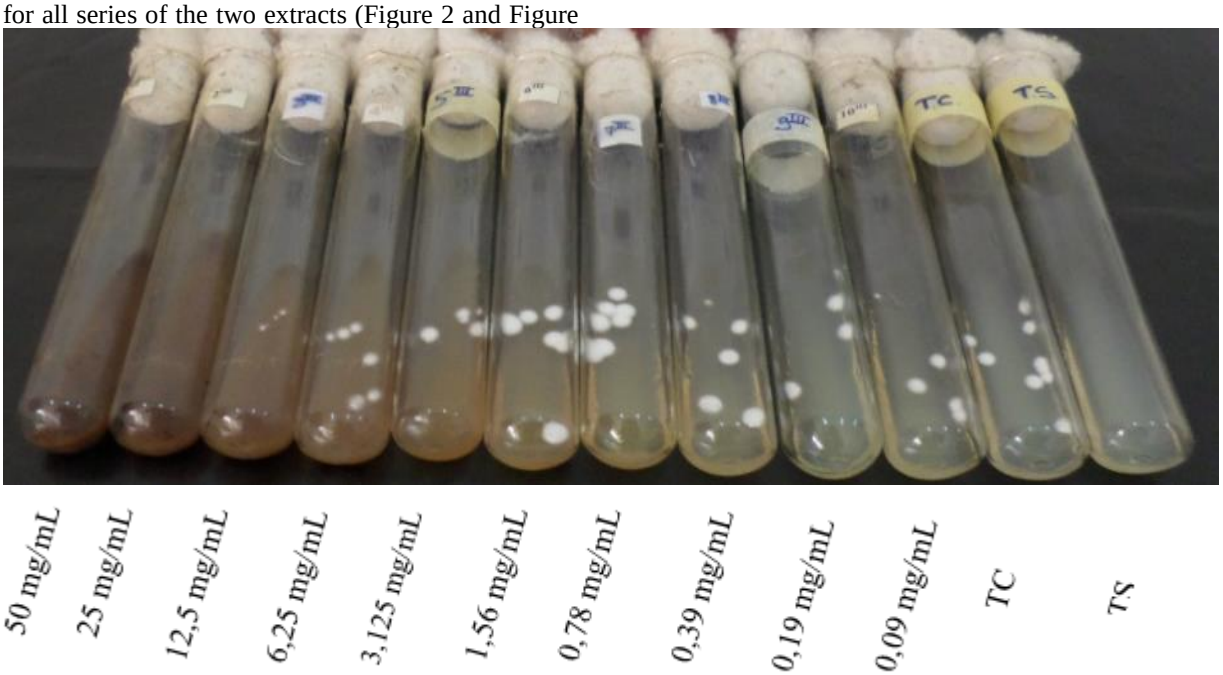

Figure 2: Dose response action of the total aqueous extract of *Hunteria eburnea* on the *in vitro* growth of *Trichophyton mentagrophytes* after 10 days of incubation.
TC: control control, TS: sterility control

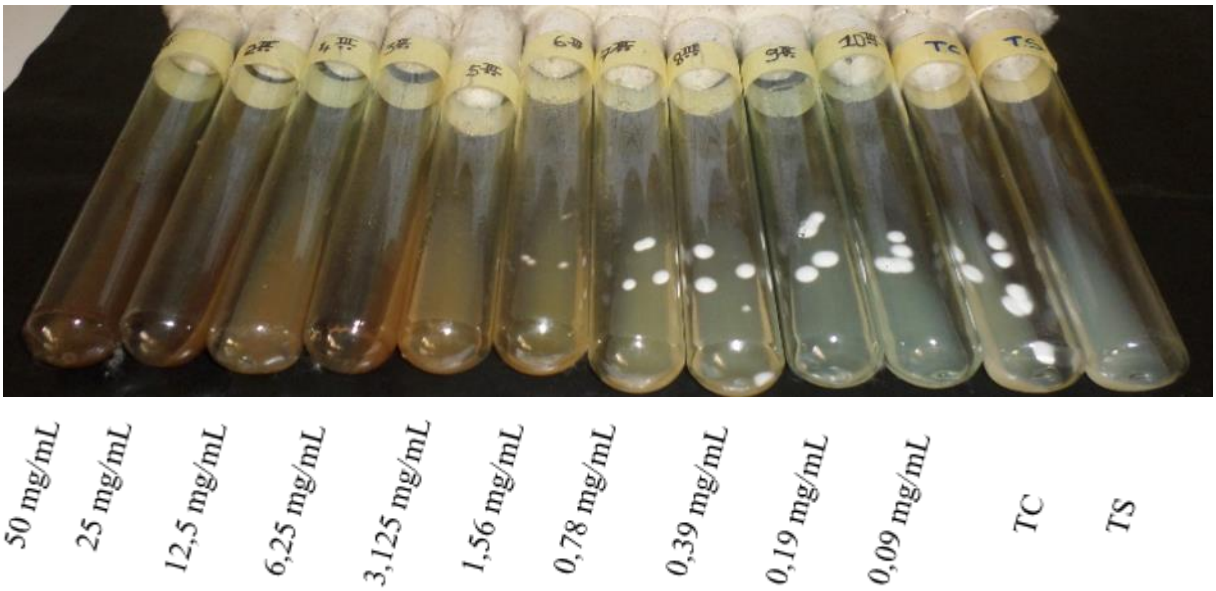

Figure 3: Action dose response of the 70% ethanolic extract of *Hunteria eburnea* on the *in vitro* growth of *Trichophyton mentagrophytes* after 10 days of incubation.
TC: control control, TS: sterility control

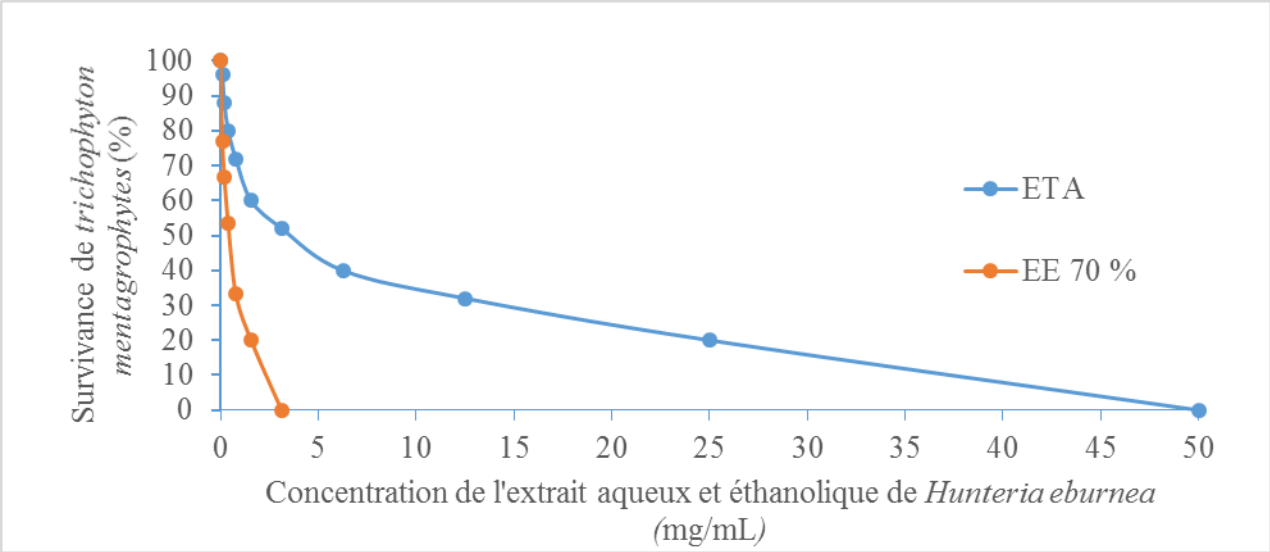

Figure 4: Sensitivity of *T. mentagrophytes* to the aqueous and ethanolic total extracts of *Hunteria eburnea*

Table I: Values (mg / mL) of the antifungal parameters of *Hunteria eburnea* extracts

souche	ETA de *Hunteria eburnea*			EE 70 % *Hunteria eburnea*		
Trichophyton mentagrophytes	MIC	MCF	IC_{50}	MIC	MCF	IC_{50}
	50	50	3	3,12	3,12	0,5

Table II: Results of the phytochemical screening of extracts of *Hunteria eburnea*.

	Composition chimique des extraits de *Hunteria eburnea*						
Extraits	Sap	Ter/Ster	T.Gall/T.Cath	Coum	Alc	Poly	Flav
ETA	+	-	-	-	-	-	-
EE 70 %	-	+	-	-	+	-	-

+: presence of the chemical group
-: absence of the chemical group
Sap: saponins; Flav: flavonoids; Terp / Ster: Terpenes / Sterols; T. Gall: gall tannin; T. Cathé: catholic tannin; Coum: coumarins; Alc: alkaloids; Poly: polyphenol
ETA: aqueous total extract; EE 70%: ethanol extract 70%

4. DISCUSSION:

The study on the effects of the different extracts (total aqueous and ethanolic 70%) of *Hunteria eburnea* on the *in vitro* growth of *Trichophyton mentagrophytes* revealed a fungicidal activity of these extracts at different concentrations. *Trichophyton. mentagrophytes* is sensitive to 70% ethanolic extract and *Hunteria eburnea* extract in a dose-response relationship. The efficacy ratio established on the basis of the MCF values shows that the ethanolic extract is 16 times more active than the aqueous extract. A difference in composition between the two extracts, related to the mode of extraction, could explain these results [7]. This observation is supported by several studies that have shown that ethanol allows a better concentration of active ingredients [16-17]. In fact, according to these authors, when we go from the total aqueous extract to the ethanolic extract, some chemical groups are eliminated and others are concentrated. Also, the large groups of chemical molecules that are the terpenes and the alkaloids present in the 70% ethanolic extract would be at the origin of this strong activity. Our results are in agreement with that of [18] who showed that the presence of triterpernes and sterols gives plants antifungal properties.

5. CONCLUSION:

Antifungal tests showed that 70% aqueous and ethanolic extracts exerted antifungal activity with better activity for the ethanolic extract. This extract could therefore be an alternative to some synthetic additives. But these are only crude extracts containing a large number of bioactive compounds. It is therefore possible that they contain compounds which, once purified, could have an even better activity.

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