

Original Research Article

Botanical Study and evaluation of the antifungal activity of the 70% ethanolic extract of *Hunteria eburnea* Pichon (Apocynaceae)

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Abstract: In developing countries infectious diseases constitute a public health concern. The objective of this study is to assess the antifungal activity of the stem bark of *Hunteria eburnea*, a medicinal plant frequently used in several therapeutic formulae in the region of Haut Sassandra (Ivory Coast) against microbial diseases. An ethnobotanical survey in the region of Haut Sassandra allowed us to select *Hunteria eburnea*, one of the most used species in the treatment of skin diseases. In order to learn more about this plant and identify it well on the field, we have, in this study, made its botanical description and an evaluation of its antifungal activity against *Candida albicans*, *Cryptococcus neoformans* and *Trichophyton mentagrophytes*. This study shows that *Hunteria eburnea* has an in vitro antifungal activity against *Cryptococcus neoformans* (MFC = 6.25 mg/mL), *Candida albicans* (MFC = 25 mg/mL) and *Trichophyton mentagrophytes* (MFC = 3.12 mg/mL). *Trichophyton mentagrophytes* with (MFC = 3.12 mg/mL; IC₅₀ = 0.5 mg/mL) is the most sensitive fungal germs with 70 % ethanolic extract of the stem bark of *Hunteria eburnea*, which justifies the use of its bark in traditional environment.

Keywords: Antifungal activity, *Cryptococcus neoformans*, *Candida albicans*, *Trichophyton mentagrophytes*, plant extracts, *Hunteria eburnea*.

INTRODUCTION

In developing countries, infectious diseases are a major public health concern because of their frequency and severity [1]. Indeed, they are the source of more than 17 million deaths per year worldwide of which more than half comes from the African continent alone [2]. Various fungi including *Candida albicans*, *Cryptococcus neoformans* and *Trichophyton mentagrophytes* are involved in these infections. These diseases are more and more difficult to eradicate and their frequencies are constantly increasing [3, 4]. This is for our developing countries a real concern. Because of the constantly high cost of available drugs associated with the emergence of multi-resistant microbes, there is a renewed interest in the pharmacopoeia [5-7]. In order to help people we undertook an ethnobotanical survey in the Haut Sassandra region on plants used in the treatment of microbial diseases. At the end of the investigation, *Hunteria eburnea* Pichon (Apocynaceae) an Ivorian medicinal plant used in traditional environment against skin infections has been selected for an evaluation of its antifungal activity to provide a scientific explanation for its use.

MATERIAL AND METHODS

Material

Vegetal material

The plant material used consists of *Hunteria eburnea* trunk bark harvested in the Departement of Issia (Ivory coast) in August 2015. The name of the plant was done in the vegetal identification center of the University Felix Houphouët Boigny of Ivory Coast. The identification number is: 01/06/1981-Ake Assi 15904, Forest of Banco.

Microbial material

The strains such as: *Trichophyton mentagrophytes*, *Cryptococcus neoformans* and *Candida albicans* on which we worked, were provided to us by the Mycology Laboratory of Faculty of Medical Sciences of the Felix Houphouët Boigny University (Abidjan, Ivory Coast).

Methods

Monographic study of Hunteria eburnea

For easy identification of this plant in natural environment, a complete monographic study was conducted.

It took into account:

- the botanical family of the plant ;
- Bibliography of this botanical family ;
- a detailed description of the plant ;
- the geographical distribution of this plant ;
- some traditional therapeutic uses in the West African pharmacopoeia.

Preparation of extracts

Hunteria eburnea stem bark harvested, were cut, rinsed with water and dried in the sun. These dried plant parts were then reduced to a fine powder with an electric grinder-MAG IKA RTC. We obtain a grey powder. The 70 % ethanolic and total aqueous extracts were prepared according to the method described by Zirihi and Kra [8].

• Total aqueous extract

One hundred grams (100 g) of powdered bark were homogenized in 1 liter of distilled water in a Blender (Mixer) Life's Superb brand (LS-317) for three minutes at room temperature. This operation is repeated three times and the homogenate obtained is filtered successively on hydrophilic cotton and then on paper Wattman (3 mm). Using an oven controlled at 50 °C, the extraction solvent is eliminated. The powdered extract obtained after evaporation and drying, constitutes the total aqueous extract (ETA).

• 70 % ethanolic extract

Five grams (5 g) of ETA were dissolved in 100 mL of 70 % ethanol and then homogenized in a blender. After decantation in a separatory funnel, the collected supernatant is filtered through cotton to get rid of any residue and dried in an oven (50 °C). The powder obtained constitutes the 70 % ethanolic extract (EE 70 %).

Yield calculation

The yield is the quantity of extract obtained from the plant powder. It is expressed as a percentage. In practice, it has been determined by the ratio of weight of the solids content after evaporation on the weight of the dry plant material powder used for the extraction, multiplied by 100. This results is indicated by the following formula:

$$Yd = (m \times 100)/M$$

Yd : Extraction yield in percentage

m : mass in grams of the dry extract

M : mass in grams of the drug powder.

Preparation of culture medium

Sabouraud agar was prepared according to the manufacturer's recommendations. The incorporation of the 70 % ethanolic extract was made according to the method of double dilution with tilting tubes.

We used a series of 12 test tubes in which 10 test tubes (containing the plant extract) and 2 control tubes (one without plant extract was used as germs growth control, the other germ and without plant extract was used as a sterility control light of the culture medium). For the 10 test tubes, the range of concentration was 50 mg/mL to 0.098 mg/mL (in a geometric bond due 1/2). All twelve tubes of each series (on series for each germ) were sterilized by autoclaving at 121 °C for 15 min and then inclined to the temperature of the room to allow cooling and solidification of the agar [9].

Antimicrobial test

The culture of germs on the previously prepared media was made by culturing 1000 cells equivalent to 10 µL of a suspension 10⁻¹ of:

- Culture of 3 days of incubation of *Candida albicans* ;
- Culture of 5 to 10 days of incubation of *Trichophyton mentagrophytes* ;
- Culture of 3 days of incubation of *Cryptococcus neoformans*.

After incubation at 30 °C, colonies of different germs in each series were counted and the growth in the 10 tubes measured in the percentage of survival was calculated based on 100 % survival in control tube of growth [10]. The method of calculating the percentage of survival was done using the following formula:

$$S = (n/N) \times 100 \text{ [11]}$$

S = Survival percentage germs

N = number of colonies in the control tube

n = number of colonies in the test tube.

Antifungal parameters research

Data processing has identified the following antifungal parameters:

- The MIC (Minimum Inhibitory Concentration) : it's the concentration of extract in the tube for which no visible growth was observed with the naked eye ;
- The IC₅₀ (concentration for fifty percent inhibition) : it's the concentration that gives 50 % inhibition. It is determined graphically from the representation of the sensitivity curves of the extracts of *Candida albicans*, *Trichophyton mentagrophytes* and *Cryptococcus neoformans* ;
- The fungicide (CMF minimum fungicidal concentration) after 72 hours of incubation, the surface of the agar contained in test tubes having resisted the germs is slightly taken, cultured with a platinum loop on neutral agar then incubated for 72 hours at room temperature. Two cases are possible:
 - If there is presence of colonies, the extract is said fungistatic.

- If there is no colonies, the extract is said fungicide. CMF is thus determined (Concentration Minimum Fungicide giving 99.99 % inhibition compared to control growth control tube).

RESULTS

Monographic study of the plant

Hunteria eburnea belongs to the family of Apocynaceae :

- Apocynaceae family

Apocynaceae are currently the subject of a large number of botanical, chemical and pharmacological studies worldwide because many species are rich in alkaloids or Heterosides having high physiological activity.

These are woody liana, shrubs, bushes or trees, rarely herbs. The species has intire simple, opposite or whorled, without stipules. The inflorescences are usually cymose. The fruits are berries, drupes, capsules or follicles. Flora of Ivory Coast has 74 species of Apocynaceae [12].

- *Hunteria eburnea* Pichon

Common name Bete : Krigbiyi

- **Description**

Hunteria eburnea is a shrub or small tree with a very thin smooth bark, brown notch, white latex. The leaves are simple, opposite, elliptic to oblong glabrous, there are 12 to 24 pairs of ribs. The inflorescences are small terminal or axillary cymes. The flowers are fragrant white from December to April. The fruits are globular linked, orange yellow in maturity with diameter of approximately 5 cm. In the fruit, there is from 10 to 12 seeds buried in a gelatinous pulp. It is a species of wet forests underwood (Figure 1).

- **Geographical distribution**

This species is represented in African rain forests.

- **Therapeutic use**

The stem bark decoction is used after drink against microbial diseases in the region of Daloa (Ivory Coast) and against malaria.

In Sierra Leone, is prepared with the bark of *Hunteria eburnea* a bitter decoction is used as a stomachic and lotion to treat fever.



Fig-1: the figure shows the leafy branch of *Hunteria eburnea* (photo Kanga, 2014)

Yield of differents extracts

We obtained from 200 g of powder, 20 g of total aqueous extract a yield of 10 % and from 5 g of total aqueous extract, we got 2 g of 70 % ethanolic extract or a yield of 40 %.

Antimicrobial test

After three days of incubation at 30 °C for *Candida albicans*, *Cryptococcus neoformans* and five days of incubation at 30 °C for *Trichophyton mentagrophytes*, compared to the control was observed a gradual decrease in number of colonies of *Candida albicans*, *Cryptococcus neoformans* and *Trichophyton mentagrophytes* as and as concentrations of the extract increased in the experimental tubes.



C₁ C₂ C₃ C₄ C₅ C₆ C₇ C₈ C₉ C₁₀ T_c T_s

Fig-2: the figure shows the action dose response of the 70 % ethanolic extract of *Hunteria eburnea* on the *in vitro* growth of *Candida albicans* after 72 hours

Concentrations ranging from C₁ = 50 mg/mL to C₁₀ = 0.098 mg/mL

T_c = control tube, T_s = sterility tube



C₁ C₂ C₃ C₄ C₅ C₆ C₇ C₈ C₉ C₁₀ T_c T_s

Fig-3: the figure shows the action dose response of the 70% ethanolic extract of *Hunteria eburnea* on the *in vitro* growth of *Trichophyton mentagrophytes* after 10 days

Concentrations vary from C₁ = 50 mg/mL to C₁₀ = 0.098 mg/mL

T_c = control tube, T_s = sterility tube



C₁ C₂ C₃ C₄ C₅ C₆ C₇ C₈ C₉ C₁₀ T_c T_s

Fig-4: the figure shows the action dose response of the 70% ethanolic extract of *Hunteria eburnea* on the *in vitro* growth of *Cryptococcus neoformans* after 3 days

Concentrations ranging from C₁ = 50 mg/mL to C₁₀ = 0.098 mg/mL

T_c = control tube, T_s = sterility tube

Antifungal parameters

Experimental data translated as curves are summarized in figure 5. The values of the antifungal parameters of 70 % ethanolic extract for the three fungal strains (IC₅₀ : Concentration for 50 % inhibition, CMF : Minimum Concentration fungicidal and CMI : minimum inhibitory Concentration) determined are

shown in table I. In general, all curves have a decreasing pace with variable value slopes.

Trichophyton mentagrophytes has a steep slope (near the axis of ordinates) as *Cryptococcus neoformans* with an average grade. As to *Candida albicans*, it has a slope a little further away from the axis.

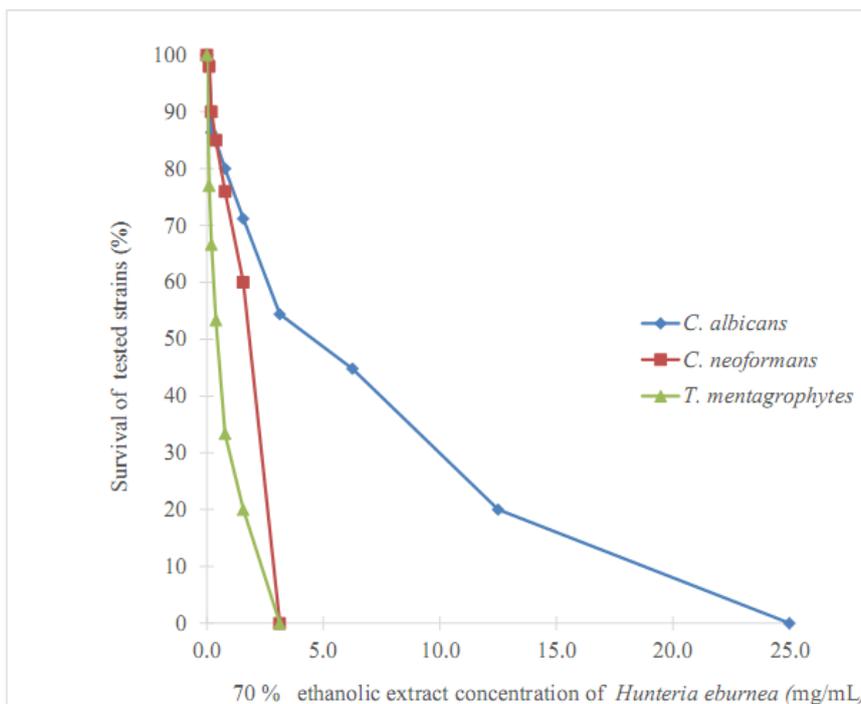


Fig-5: the figure shows the sensitivity of *C. albicans*, *T. mentagrophytes* and *C. neoformans* in 70 % ethanolic extract of *Hunteria eburnea*

Table 1 : The table shows the values of antifungal parameters of 70 % ethanolic extract of *Hunteria eburnea*

70 % ethanolic extract of <i>Hunteria eburnea</i>	Antifungal parameters (mg/mL)	Strains		
		<i>C. albicans</i>	<i>C. neoformans</i>	<i>T. mentagrophytes</i>
	CMI	25	3,12	3,125
	CMF	25	6,25	3,125
	CI ₅₀	4,6	1,8	0,5

DISCUSSION

This study aimed to evaluate the antifungal activity of 70 % ethanolic extract of *Hunteria eburnea* stem bark on the *in vitro* growth of *Candida albicans*, *Cryptococcus neoformans* and *Trichophyton mentagrophytes*.

The results have shown the sensitivity of strains and to determine the different antimicrobial parameters that are IC₅₀, CMF and CMI. These results indicate that the three fungal strains are tested, are sensitive to the 70 % ethanolic extract. *Trichophyton mentagrophytes* was the most sensitive to the ethanolic extract with a very low IC₅₀ value (0.5 mg/mL), while *Candida albicans* was the least sensitive. Indeed for each strain, this sensitivity is reflected by a decrease in

the number of colonies in each tube with increasing concentration (Figure 2, 3, 4).

The declining lines of all the sensitivity curves clearly shows that the extract is active according to dose-response link (Figure 5). Indeed one extract has a very high activity when the value of CMF is between 6.25 and 0.780 mg/mL and a average activity when the value of CMF is between 50 mg/mL and 6.25 mg/mL [13]. The CMF of *Trichophyton mentagrophytes* being equal to 3.125 mg/mL it can be said that 70 % ethanolic extract had strong activity on *Trichophyton mentagrophytes*. As for *Cryptococcus neoformans* and *Candida albicans*, the ethanolic extract had an average activity (CMF equal 6.25 and 25 mg/mL respectively for *Cryptococcus neoformans* and *Candida albicans*).

The CMF of 70 % ethanolic extract equal to 25 mg/mL of *Candida albicans* is similar to Kra who worked on the same plant with the same germ [14]. Indeed Kra showed that *Candida albicans* was less sensitive to 70 % ethanolic extract. The antifungal activity of stem bark could be explained by the fact that this species would be richer in anti-infectious active principle. Biologically active substances of *Hunteria eburnea* stem bark have a mechanism of action enabling them to reach and effectively deteriorate vital targets within fungal cells. The extraction method helps to well concentrate active principle in the ethanolic extracts [8].

Antifungal parameters values shown in Table II and Figure 5 summarized all the sensitivity curves of *Candida albicans*, *Cryptococcus neoformans* and *Trichophyton mentagrophytes* to the 70 % ethanolic extracts confirm the use of this plant in traditional environment as an anti-infectious [15, 16].

CONCLUSION

This study places us on the anti-infectious therapeutic benefits granted to the traditional environment in this plant species. This plant confirms the real potential of antidermatophyte of *Hunteria eburnea*. In this study, we can say that the tested strains are sensitive to the extract in a dose-response relation. We might also remember that the use in traditional environment of this plant as an antimicrobial ones is justified. Further analysis by phytochemical sorting followed by chromatography on thin layer and column, will allow us to isolate the different molecules in the EE70 % of *Hunteria eburnea* to clarify the nature of the molecules with antifungal activity.

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