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Evaluation of anti-fungal activity of fractional extracts of *Hugonia platysepala* on the germs responsible for candidiases in HIV-infected people

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ABSTRACT

Candidiases are opportunistic AIDS diseases that result in high mortality and morbidity rates among people living with HIV. Their treatment is particularly difficult because of the resistance, toxicity and high cost of conventional anti-fungals. Facing this problem of treating these candidiases in the context of poverty, this study was conducted with the aim to look into the traditional pharmacopoeia plants with anti-fungal properties capable of treating them effectively and at a low cost. For this purpose, four fractions of the alcoholic extract of *Hugonia platysepala* were extracted and tested *in vitro* on *Candida albicans* resistant strain by the method of double dilution and diffusion in solid medium. The fraction of acetate (Face) was the most active with a minimal inhibitor concentration (MIC) of 0, 78 mg/ml, a minimal fongicide concentration (MFC) of 25 mg/ml and a diameter of inhibition of 13 mm which is higher than the diameter of amphotericin B and fluconazole. Phytochemical screening revealed that this Face fraction of *H. platysepala* contains terpenes, polyphenols and tannins that are believed to be responsible for its anti-fungal pharmacological properties. Thus, the use of this plant in traditional medicine is an asset in the treatment of certain candidiases including those that affect immuno-suppressed.

INTRODUCTION

Since the advent of AIDS, opportunistic infections have been resurging in our developing countries in HIV-infected people. Among them, candidiasis, with a prevalence of 18% in Africa and causing about 700,000 deaths per year in the world, represent today a real public health problem (WHO, 2017). This growing mortality is due to the problem of the care of people living with HIV (PLHIV) in poor countries, since the conventional anti-fungals are out of reach of our populations. In fact, patients from our region are unable to treat themselves because of the high cost of conventional anti-fungals, which are often ineffective and unavailable (Luce and Raffi, 2001). Also, some studies have shown that fungal germs have shown resistance to azole anti-fungals (Ackah et al, 2016).

Therefore, it is necessary to find an alternative of new molecules more adapted and less expensive in our floristic heritage. This is why, our interest for *Hugonia platysepala*, lianacent shrub of the family of Linaceae, used in traditional Ivorian medicine for its anti-fungal virtues such as: dermatoses, chronic coughs, gastroenteritis, pulmonary infections (Togola et al., 2005).

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Figure 1. *Hugonia platysepala* plant.

The present study, which aims to find a more effective phyto-molecule than conventional anti-fungals, is therefore a contribution to the fight against opportunistic candidiasis in HIV-infected individuals. It will therefore consist in determining the anti-fungal activity of the fractions of the total hydro-alcoholic extract of the leaves of *H. platysepala*, comparing them with reference anti-fungals and finally identifying by screening and by chromatography, the anti-fungal biomolecules contained in the active extracts.

MATERIALS AND METHODS

Plant material

The plant material consists of *H. platysepala* leaves harvested in the west central region of Côte d'Ivoire, whose geographical coordinates are: 5° 47' 08' North - 6° 36' 29' West, Altitude: 134 m. This plant has been authenticated at the National Floristic Center at the University Felix Houphouet Boigny Cocody (Figure 1).

The leaves were then washed, dried and crushed by using an IKA-MAG grinder. The fine powder obtained was stored in glass jars.

Fungal material

The isolates in this study were *C. albicans* susceptible strain MY18-22998 and *C. albicans* resistant strain MY18-53502. They were isolated and identified from samples of patients with HIV/AIDS at the Mycology Laboratory of the Pasteur Institute in Ivory Coast.

Extraction with organic solvents with increasing polarity

From the hydro-alcoholic extract of *H. platysepala*, fractionations were carried out to obtain the different fractions of the studied plant. The various fractionated extracts were obtained by increasing polarity extraction using four solvents as followed: hexane, dichloromethane, ethyl acetate and water. This method has been described by Goly et al. (2015).

Twenty (20) g of the hydro-alcoholic extract (ETOH) of the plant are dissolved and macerated in 150 ml of distilled water and 150 ml of hexane contained in an Erlenmeyer flask. After decantation, the supernatant is filtered. This supernatant is the hexane fraction (Fhex). After the extraction with hexane, the aqueous phase is

taken up three times according to the same method with 150 ml of dichloromethane then 150 ml of ethyl acetate to obtain respectively the dichloromethane fraction (Fdcm), the ethyl acetate fraction (Face) and the final aqueous fraction (Faqf). The fractionated extracts, thus obtained, were dried and then dissolved in dimethylsulfoxide (DMSO), which has no anti-fungal power and is the solvent used by the majority of the authors.

Preparation of the dilutions of the different split extracts for anti-fungal tests

The incorporation of the fractionated extracts Fhex, Fdcm, Face and Faqf into Sabouraud-chloramphenicol medium was made according to the double dilution method in inclined tubes described by Orsot et al.(2016).

For each of the fractions, a series of 12 test tubes were prepared comprising 10 test tubes and 2 control tubes, one without a plant extract constituting the fungal growth control and the other without extract and without germs serving as a sterility control test of Sabouraud-chloramphenicol medium. The test tubes then contain a range of decreasing concentrations of the extracts ranging from 100 to 0.195 mg/ml according to a geometric connection of 1/2 reason. After autoclaving at 121°C for 15 min, the test tubes are allowed to stand at room temperature to allow cooling and solidification of the Sabouraud-chloramphenicol agar containing the plant extracts.

Tests for determination of anti-fungal parameters

The determination of the anti-fungal parameters was made by the dilution method in agar medium described by Ambe et al. (2016) and Zirihi et al.(2007).

To revive the fungal strains, a young yeast colony was removed using a platinum loop and homogenized in 10 ml of sterile distilled water to obtain the mother suspension (10^0) concentrated at 10^6 cells/ml. From suspension 10^0, a second suspension (10^{-1}) was prepared by dilution to 1/10th of the first one to obtain a concentration of 10^5 cells/ml. This suspension is used to inoculate all prepared test tubes except the sterility control. The tubes are then incubated at 37°C for 48 h and counted by direct count. For more reliability, the tests were repeated three times. We observe the growth of the germs then we determine the percentages of survival (S):

$$S = 100 \times n/N$$

Where: S, percentage of survival; n, number of colonies in the test tube; N, number of colonies in the control tube.

The treatment of these experimental data makes it possible to determine the anti-fungal parameters: MIC, 50% inhibitor concentration (IC_{50}) and MFC:

- MIC is the minimum concentration for which there is no visible growth with the naked eye.
- IC_{50} is the concentration for 50% inhibition which is graphically determined from the sensitivity curve.
- MFC is the lowest concentration from which there is no re-growth of fungal growth. It is determined from a sub-culture carried out on new agar from the tubes in which no growth was observed.

Efficiency tests for confirmation of anti-fungal activity

After determining the anti-fungal parameters, tests were carried out to confirm the fractionated extracts anti-fungal activity. These tests were carried out by the method of diffusion in solid medium from soaked disks, used by Traoré et al. (2012) and Haddouchi et al. (2016).

The prepared inoculum (10^6 cells/ml) is deposited and dried on the surface of Sabouraud-chloramphenicol agar plate-cast. Sterilized 6 mm diameter disks, cut from Whatman paper, are soaked with extracts and placed in the inoculated Petri dishes next to control disks soaked with amphotericin B and fluconazole. Amphotericin B and fluconazole are purified reference anti-fungals with concentrations of 100 µg/ml and 25 µg/ml, respectively. While the fractionated extracts of 50 mg/ml concentration have not been completely purified.

After 48 h of incubation at 37°C, we measured the inhibition diameters observed around each soaked disk.

Phytochemical screening

After obtaining the different fractions, a phytochemical screening was carried out. This phytochemical screening was carried out in order to detect the large chemical groups contained in the Fhex, Fdcm, Face and Faqf fractions of *H. platysepala*. The summary of these staining and precipitation reactions is contained in Table 1 (Harbone, 1998).

RESULTS

Determination of the anti-fungal parameters of the fractions

After 48 h of incubation at 37°C, in a dose-dependent manner, the hexane, dichloromethane, ethyl acetate and aqueous final fractions of *H. platysepala* decrease the number of *C. albicans* colonies. The growth of the germ in the tubes, expressed as percentage of survival (S), is represented in the form of sensitivity curves in Figure 2.

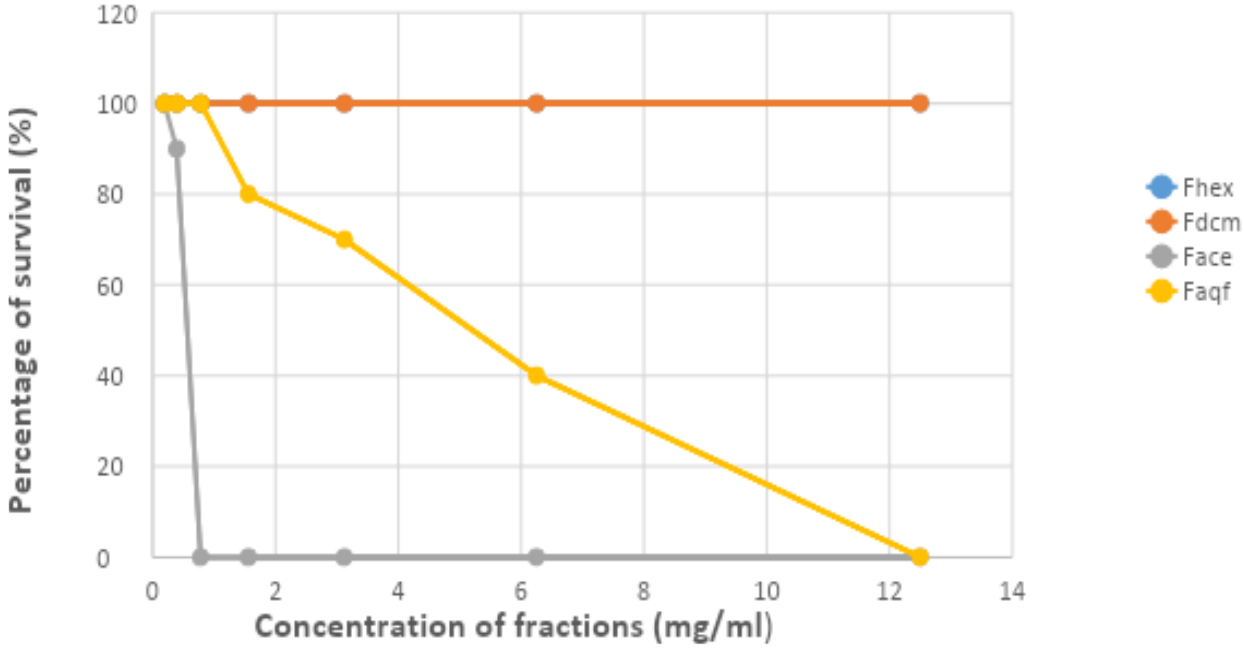

Figure 2. *C. albicans* sensitivity curves at the Fhex, Fdcm, Face and Faqf fractions of *H. platysepala*.

Table 1. Chemical group identification reaction table.

Chemical group	Reagents	Results
Sterols and polyterpenes	Acetic anhydride Sulfuric acid	Purple or purple ring
Alkaloids	Alcohol at 60°C Reagent from Dragendorf Bouchardat reagent	Orange coloring Brown-reddish color
Polyphenols	Alcoholic solution of ferric chloride 2%	Blackish-blue coloring
Flavonoids	Concentrated hydrochloric acid Magnesium chips	Red or orange color
Anthocyanins	Hydrochloric alcohol Isoamyl alcohol	Cherry red coloring Red-brown color
Gallic tannins	Stiany's reaction Ferric chloride 1%	Blue-black color
Quinones	1% NaOH	Yellow or red color

The sensitivity curves obtained show a decreasing trend, reflecting a clear anti-fungal activity of the fractionated extracts. The curves of the fractions Face and Faqf cut the abscissa axis at 0.78 mg/ml and at 12.5 mg/ml, which respectively correspond to the MIC of the Face and Faqf on *C. albicans*. On the other hand, the fractions Fhex and Fdcm which do not cut the abscissa axis do not have a MIC. The sensitivity curves also made it possible to determine the IC50s of the different active fractions (Table 2).

Fraction efficiency tests

After 48 h of incubation of the disks soaked with amphotericin B and fluconazole, and of the hexane, ethyl acetate, dichloromethane and aqueous final fractions of *H. platysepala*, in Petri dishes containing sensitive or

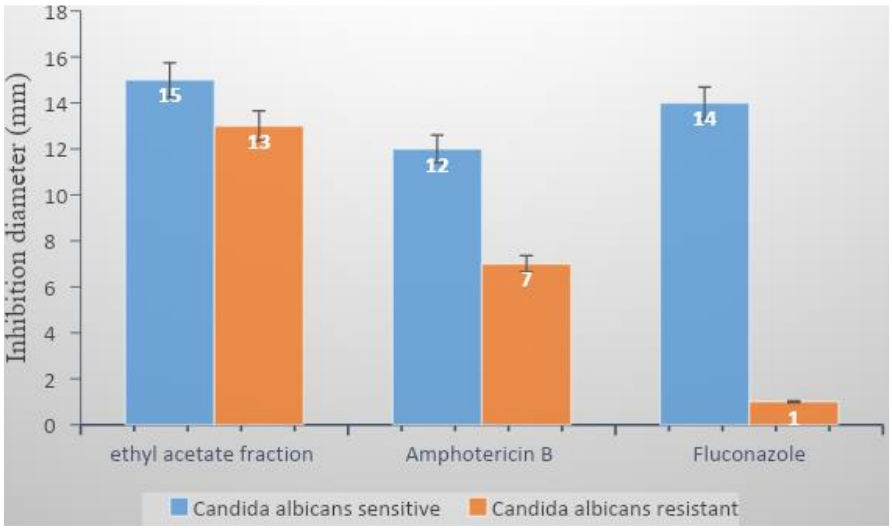

Figure 3. Inhibition of *C. albicans* sensitive and resistant by the ethylacetate fraction of *H. platysepala* and reference anti-fungals.

Table 2. *H. platysepala*'s anti-fungal parameters on *C. albicans*.

***H. platysepala* fractions**	**Anfungal parameters (mg/ml)**			**Fungicidial**
	MIC	IC_{50}	MFC	
Face	0.78	0.55	25	Fungicide
Faqf	12.5	5.1	25	Fungicide

Table 3. Identification of chemical groups in *H. platysepala* fractions.

Chemical groups	**Fhex**	**Fdcm**	**Face**	**Faqf**
Sterols and terpenes	+	-	+	+
Alkaloids	-	-	-	-
Polyphenols	-	+	+	+
Flavonoids	-	-	-	-
Anthocyanins	-	-	-	-
Tannins	-	-	+	-
Quinones	-	-	-	+

resistant strains of *C. albicans*, it is observed in the different petri dishes, the presence of inhibition halo around the soaked disks. These diameters of inhibition were measured and represented in the diagrams of Figure 3.

Sensitive and resistant strains of *C. albicans* exhibited sensitivity for Face fraction of *C. albicans* with respective inhibition diameters of 15 and 13 mm. On the other hand, it has resistance for amphotericin B and fluconazole with diameters of 12 and 14 mm respectively for the sensitive strain of *C. albicans* and also 7 and 1 mm for the resistant strain of *C. albicans*.

Phytochemical Screening of Fractions

The qualitative phytochemical study made it possible to detect the different chemical families in the fractions obtained from the hydro-alcoholic extract of *H. platysepala*. The results obtained by coloring, precipitation and UV observations are shown in Table 3.

The fractions of the *H. platysepala* leaf extract that are

the richest in molecular groups are the Face fraction and the Faqf fraction which contain Sterols, terpenes, polyphenols, tannins and quinones. The Fhex and Fdcm fractions are the poorest in chemical groups. Also none of the fractions contain alkaloids, flavonoids and anthocyanins.

DISCUSSION

The anti-fungal tests carried out in solid medium on the Fhex, Fdcm, Face and Faqf fractions of *H. platysepala* made it known that only the Face and Faqf fractions have anti-fungal activities on *C. albicans* with respective MICs of 0.78 mg/ml and of 12.5 mg/ml and a MFC of 25 mg/ml. Face and Faqf fractions, therefore, have a fungicidal action on *C. albicans*.

The work of Bagré et al. (2011) on the anti-fungal activity of *Saba comorensis* leaves on *C. albicans* showed that the ethyl acetate (Eac) extract has an IC_{50} of 4.5 mg/ml. Compared with our results, that is to say the Face fraction of *H. platysepala*, the following efficiency ratio is obtained:

CI_{5O} (Eac)/CI_{5O} (Face) = 4.5/0.55 = 8.18.

This means that the Face fraction of *H. platysepala* is eight (8) times more active than the Eac extract of *S. comorensis*. This could be explained by the fact that the bioactive compounds contained in the Face fraction were purified and concentrated. Indeed, the action of delipidation of hexane and dicloromethane solvents which preceded the ethyl acetate would have contributed to the elimination of molecules likely to prevent any fungicidal action of the active ingredients. Thus ethyl acetate has allowed a good extraction of bioactive compounds previously cleared of bulky substances. This is in agreement with the work of Kechar and Hellal (2017) who showed that it is the *ballotahirsuta* ethyl acetate extract that has the best anti-fungal activity.

The efficacy test carried out with the solid-state diffusion method allowed us to compare the anti-fungal action of the active Face fraction of *H. Platysepala* with that of the reference anti-fungals prescribed to PLHIV with candidiases. On the sensitive strain of *C. albicans*, the inhibition diameter obtained with the Face fraction is substantially greater than that of amphotericin B (AB) and fluconazole (FCA). These data confirm that the Face fraction of *H. Platysepala* has the best anti-fungal activity against this susceptible seed of *C. albicans.*

With the resistant strain of *C. albicans*, only the inhibition diameter obtained with the Face fraction, is greater than the threshold of sensitivity. The inhibition zone of this Face fraction is much greater than that of amphotericin B and fluconazole which remained below the sensitivity threshold. Indeed, according to Biyiti et al. (2004) an extract is considered sensitive when it induces a zone of inhibition greater than 10 mm. This confirms that the Face fraction of *H. platysepala* leaves is therefore sensitive to the isolate of *C. albicans*, which is resistant to the classical anti-fungals (amphotericin B and fluconazole) commonly prescribed to AIDS patients with candidiases. Zouaoui et al (2018) and Traoré et al. (2012) obtained similar results on extracts of *Chenopodium quinoa* Wild and *Annona senegalensis* with maximum inhibition rates of 91.17% and inhibition diameters of 10-13 mm.

The activity of the ethyl acetate fraction is confirmed by the phytochemical screening which revealed the presence of polyphenols, tannins, sterols and terpenes. This is in accordance with the work of Ackah et al. (2016) and Hajoui et al. (2014) who have proved that thanks to their hydroxyl groups, the polyterpene and polyphenolic compounds have an inhibitory action on fungal germs, cause cell death by the destruction of their membranes.

Thus, after the purification of the active molecules of this *H. platysepala* ethylacetate fraction, it could give better anti-fungal parameters and be beneficial for the treatment of opportunistic mycoses because according to Erhenhi et al. (2016), medicinal plants with well-established compounds can contribute to the progress of powerful drugs.

However, carrying out a toxicological study on *H. platysepala* fractionated extracts, followed by clinical trials on PLHIV with candidiasis would make this study much more complete.

Conclusion

From this study, it can be said that the ethyl acetate fraction of *H. platysepala* has a very interesting anti-fungal activity on *C. albicans* compared to amphotericin B and fluconazole. These conventional anti-fungals have shown their limits in the treatment of opportunistic candidiases of AIDS, the use of the active molecules of this medicinal plant (*H. platysepala*) as a more effective and less expensive anti-fungal drug, could be beneficial for the treatment of opportunistic candidiases of PLHIV. Thus, the extracts from this plant could therefore be used as a traditionally improved medicine against opportunistic candidiasis.

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