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Evaluation of the Anticandidosic Activity of the Crude Aqueous and Ethanolic Extract of Eucalyptus sp, a Myrtacee From the Ivorian Pharmacopeia on the in Vitro growth of Candida Albicans, Candida Glabrata and Candida Tropicalis

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ABSTRACT

With the advent of AIDS/ HIV, we assist to a trong new outbreak of thrive's mycoses. To fight against this mycoses apparition, our research team has tested the whole crude extracts action (water and hydro-alcoholic), outlet from husks of *Eucalyptus sp* a *Myrtaceae* on the *in vitro* growth of *Candida albicans*, *Candida glabrata*, *and Candida tropicalis*. Extracts incorporation to the sabouraud's geloses has been made according to double dilution method with in slope tube.

After 48 hours of incubation at 30 °C, these extract exhibited anticandidosic activity in dose-response relationship. The hydro- alcoholic extract is the most active extract on *in vitro* growth three clinical isolate of *Candida*, with the following antifungal parameters (*C. albicans* FMC = 250 µg/mL, IC_{50} = 14.57 µg/mL, *C. glabrata* FMC = 250 µg/mL, IC_{50} = 11.90 µg/mL, *C. tropicalis* FMC = 250 µg/mL, IC_{50} = 11.15 µg/mL).

The crude extracts of *Eucalyptus sp* tested showed anticandidosic activity and this activity was more pronounced with hydro-alcoholic extract.

Keywords: Myrtaceae; anticandidosic activity; *Candida albicans*; *Candida glabrat;*, *Candida tropicalis*.

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INTRODUCTION

Disregarded by the population, medicinal plants have now become an important source in the treatment of evil.

Indeed, after a long period of brilliant scientific advances on therapeutic human placed all his hopes in sophisticated laboratories and high-tech devices, we are now witnessing a renewed interest in the remedies offered by nature; medicinal plants [1, 2]. Indeed, candidiasis have become nowadays a problem of public health. It's difficult to eradicate because of their strong growth and the high number of risk factors exhibiting at the onset of candidemia [3, 4].

They can treat diseases of all kinds such as infectious [5, 6]. This is the case of candidiasis who occupy the hospital instead a disturbing especially a patients with HIV / AIDS [7, 8].

Among these medicinal plants which were granted anti-infectious properties, figure *eucalyptus sp* plant of the family *Myrtaceae*. The species of this genus (Eucalyptus) are mostly used for the treatment of bronchitis, respiratory diseases, microbial infections in traditional medecine [9, 10, 11, 12].

In order to verify the validity of anti- infectious properties given to this plant, our research team has finally initiated this study to evaluate the anticandidosic activity of *Eucalyptus sp* extracts on *in vitro* growth of *Candida albicans*, *Candida tropicalis* and *Candida glabrata*.

MATERIALS AND METHODS

MATERIAL

Plant Material

The material used is plant powder name EUCA obtained from the bark of *Eucalyptus sp*.

Fungi tested

Fungal clinical germs tested namely *Candida albicans*, *Candida tropicalis*, *Candida glabrata* were provided to us by the Mycology and Parasitology department of the Pasteur Institute of Côte d'Ivoire. These germs were isolated from patients with superficial mycoses for *Candida glabrata* and deeper mycoses for *candida albicans* and *candida tropicalis*.

Clinical isolate of the genus Candida are opportunistic yeast-like fungi they are the source of most candidiasis and are the most frequent strains in human pathology [13,14,15]. These infections are often deadly for subjects whose immune system is very weak [16].

Culture medium

We used Sabouraud agar (Bio-RAD/Réf: 64449, Lot: 8B2212) buffered to pH 5.7 for this test. Appropriate medium and commonly used for fungi cultivating.

METHODS

Preparation of extracts

Bark from the trunk of *Eucalyptus sp* were cut, collected and dried in the shade. After drying, the pieces of this plant were finely grounded using an electric grinder followed by awarring blender. The powder obtained was coded EUCA. The total aqueous and ethanolic extracts were prepared as follows: One hundred (100) grams of EUCA were extracted by homogenization in blender with one liter (1L) of distilled water (mixer). After six cycles of crushing, the homogenate obtained was first centrifuged in a square of fabric and then filtered successively twice on absorbent cotton and once on Wattman filter paper 3 mm. The obtained filtrate was concentrated using under vaccum at 60 °C. The powder obtained is the crude aqueous extract X_{Aq}. The hydroalcoholic extract was prepared using the same method using a solvent mixture constitutes ethanol-water (v/v; 70/30). The ethanol extract obtained evaporation to dryness was coded X_0.

Both extracts were tested separately on the *in vitro* growth of *Candida albicans, Candida tropicalis* and *Candida glabrata.*

Preparation of culture medium

The medium was prepared according to the instructions of the manufacturer's protocol.

The incorporation of various plant extracts in Sabouraud agar was done by the method of the double dilution method agar slopes [17, 18, 19, 20]. For each plant extract, each series consists of 10 test tubes. Eight (8) of these tests tubes containing plant extract. And the other 2 tubes are considered as control tubes. Among these 2 tubes, one without vegetable extract was used as witness of germs growth control while the other without germs and extract was used as witness of culture medium sterility control. For 8 test tubes concentrations ranging from 2,000 to 15.62 µg/mL binding by a geometrical reason of ½.

After the inclusion of the 8 samples in test tubes, all 10 tubes of each extract were removed by the use of forceps sterilized by flaming for 15 minutes at 121 °C and then inclined to room temperature of the laboratory to cooling and solidification of the agar [17, 18, 19, 20]

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Antimicrobial Assay

Antimicrobial tests were performed using the same method for the three strains of Candida.

From young *candida* culture (48 hour of incubation), the inoculums were prepared as follows:

A young *Candida* colony taken with a handle was homogenized in 10 mL of sterilized distilled water. This gives the suspension (10^0) concentrated to 10^6 cells/mL. From this suspension, a second suspension (10^{-1}) was prepared by dilution of the first by 1/10. It charges to 10^5 cells/mL.

For each of the test tubes (except the control tube of sterility) the germs culture was done on the agar slant previously prepared medium by seeding 10 µL of the suspension 10^{-1}. This corresponded to 1,000 cells seeded. The cultures thus produced were incubated at 30 °C for 48 hours.

After incubation, the colonies of *Candida* were numbered with a pen colony counter (serial No. 23382 Scinceware of Bel-Art). Moreover, the growth in the experimental tubes was evaluated as a percentage of survival calculated compared to 100 % survival in the control tube growth control [17, 18, 19, 20]. The processing of these data was used to determine the following antifungal parameters:

- The Minimum Inhibitory Concentration (MIC) is the lowest concentration for which there is no growth visible to the naked eye.

- The Minimum Fungicidal Concentration (MFC) is the smallest extract concentration in the tube which gave 99.99 % inhibition compared to the control tube. It's obtained after transplanting the agar of the tube corresponding to the MIC.

- The Concentration for 50 % Inhibition (IC_{50}) is the concentration which gave 50 % inhibition. This parameter was determined graphically.

RESULTS

After 48 hours of incubation at 30 °C, it was observed compared with control tube, a gradual decrease in the number of colonies in all three species of *Candida* tested as the concentrations of the plant extracts in the test tube increases. These results are statistical averages of 6 experiments for each extract.

Effective inhibitions were obtained at different concentrations level. Experimental data presented as activity curves of the extract are shown in Figure 1 for *Candida albicans*, Figure 2 *Candida glabrata*, Figure 3 for *Candida tropicalis*.

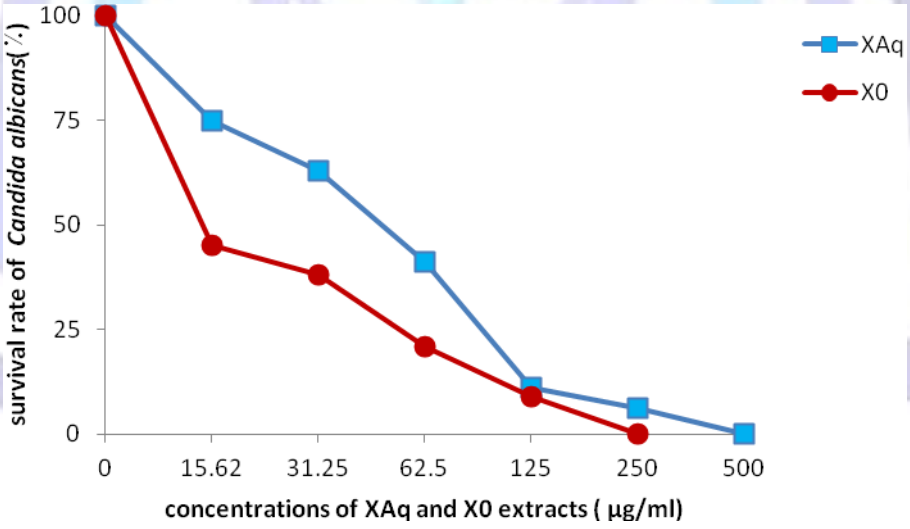

Figure 1: Antifungal Activity of X_0 and X_{Aq} on the *in vitro* growth of *Candida albicans*

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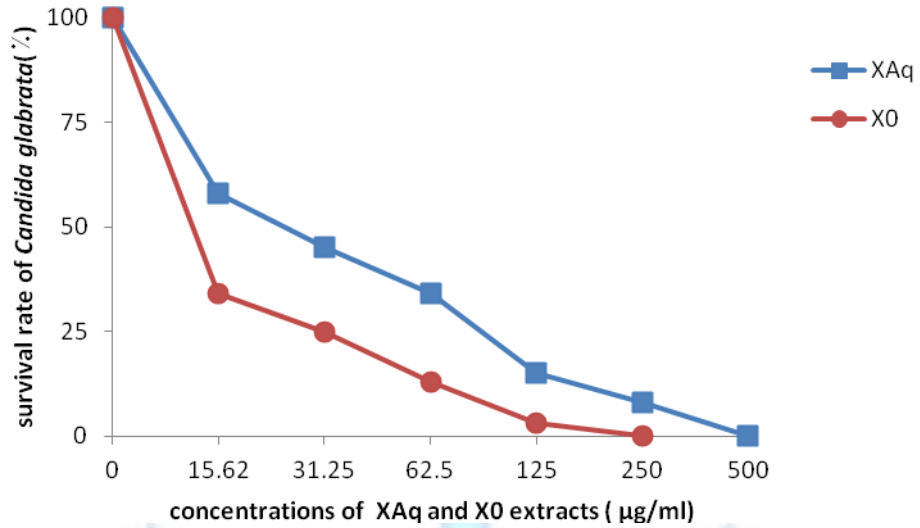

Figure 2: Antifungal Activity of X_0 and X_{Aq} on the *in vitro* growth of *Candida glabrata*

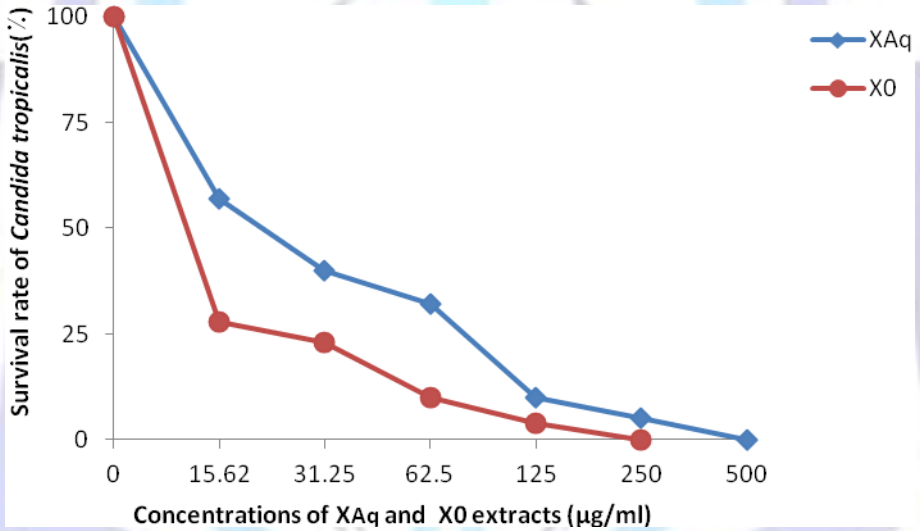

Figure 3: Antifungal Activity of X_0 and X_{Aq} on the *in vitro* growth of *Candida tropicalis*

In general, all activity curves have a decreasing pace with slopes more or less strong according to the extract. However, for the three germs tested, the activity curves of the extract X_0 present stronger slope than X_{Aq} extract (Figure 1 to 3). The values are equal to the one of the MFC. In fact, we still notice that there are not visible colonies on the agar gelose after the period incubation. Values of FMC and IC_{50} for both crude extracts are shown in Tables 1 and 2.

Table 1: Compared values of antifungal parameters of X_{Aq} extract.

Extrait	Germes fongiques	Paramètres antifongiques	
		CMF (µg/mL)	CI_{50} (µg/mL)
X_{aq}	*Candida albicans*	500	48.54
	Candida glabrata	500	24.14
	Candida tropicalis	500	21.30

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Table 2: Compared values of antifungal parameters of X_0 extract.

Extrait	Germes fongiques	Paramètres antifongiques	
		CMF (µg/mL)	CI_{50} (µg/mL)
X_0	*Candida albicans*	250	14.57
	Candida glabrata	250	11.90
	Candida tropicalis	250	11.15

DISCUSSION

The analysis of the results of antifungal tests with aqueous extract EUCA shows that *Candida albicans*, *Candida tropicalis* and *Candida glabrata* are sensitive to the extract with FMC values (MFC X_{Aq} = 500 µg/mL). No cases of resistance have been noted. The value of the inhibitory concentration obtained revealed that the X_{Aq} extract has a more or less pronounced antifungal activity.

Given the good performance obtained with the aqueous extract, we wanted to know if we could improve the activity of the extracts using a solvent for extraction. In view of the study conducted by Zirihi and Kra (2003) [17], we chose the ethanol water mixture 70/30 (v/v) as an extraction solvent.

The results of antifungal tests with the total ethanolic extract of EUCA shows that *Candida albicans, Candida glabrata* and *Candida tropicalis* are sensitive to X_0 with MFC values (MFC X_{Aq} = 250 µg/mL) . The report based on the FMC value shows that X_0 is 2 times more active than X_{Aq}. These figures allow a comparison of the performance of the 2 crude extracts.

Note that the greater the slope of a curve is high, that is to say, the more closer the slope approaches the ordinate axis, the more the extract is considered active.

- Against Candida albicans

The comparison our results to those of Zihiri and Kra in 2003 [17] reveals that the aqueous and ethanolic extracts EUCA are significantly more active than PYMI extract ($PYMI_0$, MFC = 50,000 µg/mL; $PYMI_1$, MFC = 25,000 µg/mL). In fact, X_{Aq} and X_0 are 100 times more active than those extracts. In addition, these extracts X_{Aq} and X_0 show better results than those of *Spermacoce verticillata* (hydro-ethanolic extract; MFC = 100,000 µg/mL) [21].

- Against Candida tropicalis

Comparing the performance of our X_{Aq} extract to the aqueous extract of *Borreria latifolia, Borreria verticillata, Euphorbia hirta, Vernonia colorata, Turraea heterophylla* having a value of MFC 2,000 µg/mL as shown by the work of FEZAN in 2007 [22] on both strains including *Candida albicans* we noted that EUCA is significantly more active (4 times).

Our results compared to those of KPOROU in 2009 [23] on *Candida tropicalis*, shows that the total extracts are more active than the aqueous and hydroalcoholic extracts (70 %) of *Miltracarpus scaber* (MFC X_{Aq} = 100,000 µg/mL; FMC X_0 = 6,250 µg/mL). The extracts X_0 and X_{Aq} of EUCA are respectively 200 and 25 times more active against *Candida tropicalis.*

We noted that the activity of the crude extracts, based on IC_{50} *Candida albicans* is the most resistant strain while *Candida tropicalis* is the most sensitive strain with the extract X_{Aq}. However with X_0 extract the sensitivity of *Candida glabrata* and *Candida tropicalis* are substantially the same.

CONCLUSION

This study has allowed us to highlight the anti-infectious property attributed to this plant. Total extracts of *Eucalyptus sp* tested showed good anticandidosic activity and this activity was more pronounced with the ethanolic extract. Both crude extract acts on germs of *Candida spp* in a dose response relationship. However *Candida albicans* remains the most resistant clinical isolate.

PROSPECTIVE

Looking ahead, further studies by fractioning the ethanol extract in different solvents followed by a phytochemical screening would better concentrate the active ingredient.

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