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

Agré Don Josette¹, Ackah Jacques Auguste Alfred Bognan¹,², Yayé Yapi Guillaume¹*, Kporou Kouassi Elysée², Loukou Yao Guillaume⁴ and Djanm Allico Joseph¹,³

¹Laboratoire de pharmacodynamie biochimique, U. F. R. Biosciences, Université Félix Houphouët Boigny Cocody-Abidjan, 22 BP 582 Abidjan 22 (Côte d’Ivoire)
²UFR Agroforesterie, Filière Biochimie Microbiologie, Université Jean Lorougnon Guédé, BP 150 (Côte d’Ivoire)
³Département de Biochimie Fondamentale et Clinique, Institut Pasteur de Côte d’Ivoire BP490 Abidjan 01 (Côte d’Ivoire)
⁴UFR des Sciences Pharmaceutiques et Biologiques, Université Félix Houphouët-Boigny Cocody-Abidjan, 22 BP 582 Abidjan 22, Côte d'Ivoire

*E-mail: yayeyapi@yahoo.fr

ABSTRACT

With the advent of AIDS/ HIV, we assist to a strong 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 Myrtacee 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, IC₅₀ = 14.57 µg/mL, C. glabrata FMC = 250 µg/mL, IC₅₀ = 11.90 µg/mL, C. tropicalis FMC = 250 µg/mL, IC₅₀ = 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 glabrata; Candida tropicalis.
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 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 medicine [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 antifungal 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 vacuum at 60 °C. The powder obtained is the crude aqueous extract Xaq. 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 Xe.

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].
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 ($10^6$) concentrated to $10^5$ 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 Scincwear 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 (IC50) 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](image-url)
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 X₀ present stronger slope than X₀ 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 IC₅₀ for both crude extracts are shown in Tables 1 and 2.

**Table 1: Compared values of antifungal parameters of X₀ extract.**

<table>
<thead>
<tr>
<th>Extrait</th>
<th>Germes fongiques</th>
<th>Paramètres antifongiques</th>
<th>CMF (µg/mL)</th>
<th>CI₅₀ (µg/mL)</th>
</tr>
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<tbody>
<tr>
<td>X₀</td>
<td><em>Candida albicans</em></td>
<td></td>
<td>500</td>
<td>48.54</td>
</tr>
<tr>
<td></td>
<td><em>Candida glabrata</em></td>
<td></td>
<td>500</td>
<td>24.14</td>
</tr>
<tr>
<td></td>
<td><em>Candida tropicalis</em></td>
<td></td>
<td>500</td>
<td>21.30</td>
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</tbody>
</table>
**Table 2: Compared values of antifungal parameters of X₀ extract.**

<table>
<thead>
<tr>
<th>Extrait</th>
<th>Germes fongiques</th>
<th>Paramètres antifongiques</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CMF (µg/mL)</td>
</tr>
<tr>
<td>X₀</td>
<td>Candida albicans</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>Candida glabrata</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>Candida tropicalis</td>
<td>250</td>
</tr>
</tbody>
</table>

**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 Xₐq = 500 µg/mL). No cases of resistance have been noted. The value of the inhibitory concentration obtained revealed that the Xₐq 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 Zihiri 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 X₀ with MFC values (MFC Xₐq = 250 µg/mL). The report based on the FMC value shows that X₀ is 2 times more active than Xₐq. 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 (PYMI₂, MFC = 50,000 µg/mL; PYMI₁, MFC = 25,000 µg/mL). In fact, Xₐq and X₀ are 100 times more active than those extracts. In addition, these extracts Xₐq and X₀ 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 Xₐq 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 Mitracarpus scaber (MFC Xₐq = 100,000 µg/mL; MFC X₀ = 6,250 µg/mL). The extracts X₀ and Xₐq of EUCA are respectively 200 and 25 times more active against Candida tropicalis.

We noted that the activity of the crude extracts, based on ICₐ₀, Candida albicans is the most resistant strain while Candida tropicalis is the most sensitive strain with the extract Xₐq. However with X₀ extract the sensitivity of Candida glabrata and Candida tropicalis is 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 anticandidosis 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.

**ACKNOWLEDGEMENT**

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- Member of National Laboratory of Heath public of Côte d'Ivoire.
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