Original Article

Optimization of Antifungal Activity of *Terminalia catappa* (Combretaceae) on the In vitro Growth of *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton mentagrophytes*

Ackah Jacques Auguste Alfred Bognan ¹,³, Yayé Yapi Guillaume ¹,³*, Koulibaly Annick², Agré Don Josette³, Djaman Allico Joseph ³,⁴

¹ Unité Formation et de Recherche Agroforesterie, Département de Biochimie Microbiologie, Université Jean Lorougnon Guédé, BP 150 Daloa (Côte d'Ivoire)
² Unité Formation et de Recherche Agroforesterie, Département de Botanique, Université Jean Lorougnon Guédé, BP 150 Daloa (Côte d'Ivoire)
³ Laboratoire de pharmacodynamie biochimique, U.F.R. Biosciences, Université Félix Houphouët Boigny Cocody-Abidjan, 22 BP 582 Abidjan 22 (Côte d’Ivoire)
⁴ Département de Biochimie Fondamentale et Clinique, Institut Pasteur de Côte d’Ivoire BP 490 Abidjan 01 (Côte d’Ivoire)

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**ABSTRACT**

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**Objective:** Fungal are responsible for many infectious disease today and continue spreading. The frequency and severity have been growing at an alarming rate for years despite the existence of many drugs. In view of this pathetic situation, it is necessary to develop new active molecule from medicinal plants available and used in the traditional environment. Thus our study on the antifungal activity of *Terminalia catappa* on the in vitro growth of *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton mentagrophytes* was embarked upon. **Approach experimental:** To achieve this, nine extracts comprising of, two total extracts (aqueous and hydroethanolic 70%), six extracts from liquid-liquid partition of the ethanolic extract and chromatographic fraction F₁₂ was made and tested. The tests were carried out on Sabouraud agar double dilution method in slope tubes. **Results and discussion:** All these extract tested are active, more or less pronounced on the three fungal isolates. Among these extracts, the butanolic extract (X₃.1) was the least active extract and F₁₂ fraction the most active extract. Regarding fungal isolates, *Trichophyton mentagrophytes* (MFC=0.5 μg/mL) was the most sensitive germ to F₁₂ fraction and *Aspergillus fumigatus* the least sensitive (MFC= 10 μg/mL). As for *Candida albicans*, it was inhibited by F₁₂ fraction at a value of MFC = 5 μg/mL. **Conclusion:** The chromatographic extract (fraction F₁₂) is most active extract.

**Keywords:** *Terminalia catappa*, Antifungal activity

**1. INTRODUCTION**

Plants have always been used by humans as good source of food, cosmetics and drugs. From time immemorial they formed the basis of some very developed traditional medical systems. These traditional system of medicine still plays a...
fundamental role to the extent that the World Health Organization (WHO) estimated that 80% of the world population depends on traditional medicine to solve their underlying health problems. And Terminalia catappa Linne, pantropical plant is one of the plants used in traditional medicine environment to treat many diseases. In traditional medicine, this species is known for its, antiparasitic, anti-infectious, antihypertensive and antidiabetic properties. The emergence of fungal infections has become a serious public health problem. The fungal species responsible for most infections are Candida albicans, they are responsible for most of candidiasis, Aspergillus fumigatus, is a filamentous saprophyte fungus, opportunistic responsible for most fungal infections called pulmonary aspergillosis and extrapulmonary. And Trichophyton mentagrophytes, a polymorphic fungus causing ringworms called microïdes, giving a lesion similar in appearance to kerion, difficult to treat.

In view of these properties that this plant possesses, this study was initiated to test the antifungal properties shown by this plant and improve to some extent this activity.

2. MATERIAL AND METHODS

Plant Material

The plant material is the powder obtained from the bark of Terminalia catappa it was coded TEKAM3.

Fungus tested and culture Media

The isolated fungal tested were Candida albicans, Aspergillus fumigatus and Trichophyton mentagrophytes provided by the mycology laboratory of the Training and Research Unit of the faculty of Medical Sciences of the University of Félix Houphouët-Boigny (Côte d'Ivoire). These germs were isolated from patients in the Infectious diseases department of the University Teaching Hospital of Treichville (Côte d'Ivoire).

The Sabouraud agar, acidic pH (5.7) was used for various tests.

Preparation of extracts

The bark of Terminalia catappa was cut into pieces dried and was crushed. The powder obtained (TEKAM3) was used to prepare the ethanolic extracts this as follows:

One hundred grams (100 g) of powder was extracted in 1 L Solvent (ethanol 70%). The homogenate obtained was filtered in a square of fabric and successively filtered twice on cotton wool and then Whatman 3mm filter paper. The ethanolic extract (X₀), it was obtained by evaporation to dryness.

Subsequently, three (3) portions of X₀ of 10 g each were formed and separately subjected to a liquid/liquid partition in 100 mL of 3 different solvents (hexane-water, ethyl acetate-water, butanol-water, 50/50; v/v). After decantation, the various phases were concentrated under vacuum; the following extracts were obtained respectively:

X₁: the hexane phase, X₂: the aqueous phase from the hexane – water partition, X₃: the acetate phase, X₄: the aqueous phase resulting from the partition of ethyl acetate – water, X₅: the butanol phase, X₆: the aqueous phase resulting from the partition butanol – water.

In addition, X₇ extract was then chromatographed on a gel filtration column of Sephadex G₂₅ whose characteristics are: diameter = 1 cm, gel height = 55 cm, flow = 0.22 ml / min.

The fractionation was done with 5 g of the extract X₇. Distilled water was used as eluent and 10 mL samples were taken. Twenty (20) fractions were obtained (F₁ to F₂₀).

Thus all these extracts were tested for biological assays (three germs).

Preparation of culture media

The medium was prepared according to the instructions of the manufacturer’s protocol. The inclusion of the plant extracts in the agar was made according to the method of the double dilution agar slopes. Each series of test consisted of 9 tubes containing plant extracts and 2 control tubes. The control tubes are used as control tubes in which one was without a plant extract used to monitor the growth of germs, and the other germ-free tube and without plant extract was used as sterility controls to the culture medium.

For the 9 test tubes, concentrations ranged from 1560 to 0.5 g/mL biding by a geometrical reason of ½. All the 11 tubes of each series are sterilized by autoclaving at 121 °C for 15 minutes and then inclined in a storage area to allow for cooling and solidification of the agar.

Antifungal Assay

The different inoculum was prepared from fresh cultures of 3 fungal isolates on inclined agar; 48 hours for Aspergillus fumigatus and Candida albicans; and 5 days for Trichophyton mentagrophytes. Indeed, one to two colonies was removed with a handle and then homogenized in 10 mL of sterilized distilled water (10⁶ suspension, concentrated to 10⁸ cells / mL). This has helped to prepare a second suspension 10⁴ by dilution 1/10⁶ by transferring 1 mL of 10⁶ suspensions in 9 mL of sterilized distilled water. This suspension is concentrated to 10⁹ cells/mL.

The culture of different fungal germs was done on previously prepared medium, except in the sterility control tube, by transverse striations seeding of 10 µL (1000 cells) of the suspension 10⁻¹ with the aid of a micropipette.

The cultures thus produced, all tubes were incubated in an oven at 30 °C for 48 hours for Aspergillus fumigatus and Candida albicans and 5 days for Trichophyton mentagrophytes. Moreover, the charges of each inoculum were verified by dilutions of the suspension 10⁻¹ to 10⁻⁴. These tests were repeated 6 times for the extracts X₀, X₁, X₂, X₃, and 3 times for others extracts.
**3. RESULTS AND DISCUSSION**

After 48 hours and 5 days of incubation according to the germs and at 30 °C, we observed compared to the control tube's contents, a gradual decrease of the number of colonies of different fungal isolates in experimental tubes in line with the increasing concentration of the extracts. Effective inhibitions were obtained at different concentrations level depending on the extracts. In addition, the table (1) below reflects the values of the antifungal parameters of different extracts: total, partitioned and the most active fraction.

Obtained results showed that these extracts are active on all tested fungal germs. No resistance was observed (table 1). Nevertheless this performance varies according to solvents and in a dose-response relationship.

The aqueous and ethanolic extracts showed good activity on *Candida albicans isolates*. Of these extracts the most active extracts is hydroethanoic extract, based on result it is 2, 4 and 19 times more active on *Candida albicans, Aspergillus fumigatus and Trichophyton mentagrophytes* than aqueous extract. These results are 32 times better than those obtained by Kouakou *et al.* 200711 with the aqueous extract of *Thomningia sanguinia* (MFC = 1560 µg/mL on *Candida albicans* and 12500 µg/mL on *Aspergillus fumigatus*). Moreover they are better than the results obtained by Thes *et al.*, 201112 in *Trichophyton mentagrophytes* (MFC = 390 µg/mL) with a MISCA-MATES soap made from oil extracted from *Miltracearpus scaber*, *Mareea microantha* and *Cassia alata*. The activity of the ethanolic extract (X12) of *Terminalia cattapa* compared to that of the ethanol extract of *Cassia alata* on the in vitro growth of *Candida albicans* showed that the extract X12 is more active, MFC X12 = 40 µg/mL against 312 g/mL13. It is also more active than the methanol extract of Chene-liège on *Trichophyton rubrum* and *Candida albicans* with respective MIC 50000 g/mL and 12500 g/mL according to the work of Hassikou *et al.* in 201414.

Besides the total extract (hydro-ethanolic), the extracts from the partitioned and the F12 fraction effect on the in vitro growth of these fungal isolates revealed that these extracts also have better activities. That of the F12 fraction was more active given the MFC values (table).

**Table 1: Antifungal parameters of the different extracts**

| Exacts    | *Candida albicans* | Aspergillus fumigatus | Trichophyton mentagrophytes |
|-----------|---------------------|------------------------|-----------------------------|
|           | IC50 (µg/mL)        | MFC (µg/mL)            | IC50 (µg/mL)                | MFC (µg/mL) |
| X0        | 139                 | 190                    | 14                          | 90          | 3.8 | 10 |
| X<sub>1</sub> | 59                 | 390                    | 177                        | 780         | 1.4 | 10 |
| X<sub>2</sub> | 18                 | 40                     | 6                          | 40          | 1.3 | 5  |
| X<sub>3</sub> | 38                 | 190                    | 28                         | 390         | 1.8 | 5  |
| X<sub>4</sub> | 32                 | 90                     | 25                         | 190         | 1   | 10 |
| X<sub>5</sub> | 500                | 1560                   | 10                         | 780         | 1.6 | 10 |
| X<sub>6</sub> | 59                 | 190                    | 32                         | 190         | 1.27| 5  |
| F<sub>12</sub> | 1.6                | 5                      | 1                          | 10          | 0.04| 0.5|

Furthermore, regarding the different partitions, the aqueous phases are more active than the organic phases (table 1). That signified that the active ingredient would be concentrated in those phases. The most active extract as a result of partition was the extract from the hexane-water partition (aqueous phase X<sub>12</sub>). It is 4.75, 2.25 and 38 times more active respectively on *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton mentagrophytes* than its original extract X0. The activity of the extract X<sub>12</sub> is better than that reported by Agban *et al.*, 201313, with the dichloromethane extract of *Pilostigma thomningii* (MIC = 625 µg/mL) on the in vitro growth of *Candida albicans*. The partition of hydro-ethanolic extract in different solvent mixture has contributed in improving the activity of this plant species.

Fractionation of the extract X<sub>12</sub> has produced extracts of which the most active is the extract from the F<sub>12</sub> fraction (table 1). Analysis of these results showed that this fraction is 8, 4 and 10 times more active than its original X<sub>12</sub> extract on *Candida albicans, Aspergillus fumigatus* and *Trichophyton mentagrophytes* respectively. The results of the F<sub>12</sub> fraction is better than those obtained by Kporou *et al.*, 201015, of which the most active fractions (F<sub>6</sub>, F<sub>7</sub>, F<sub>8</sub>) from an hexane extract of *Miltracearpus scaber* with a MFC value of 781 µg/mL on the in vitro growth of *Candida albicans*.

From our results, the most sensitive fungal isolate is *Trichophyton mentagrophytes* and the least sensitive is *Aspergillus fumigatus, Candida albicans* has an intermediate
sensitivity. According to the MFC values, the most active extract is the F₁₂ fraction. Moreover, we observed that the filtration chromatography on Sephadex G₂₅ gel has greatly improved the antifungal activity of *Terminalia catappa*.

4. CONCLUSION

This study confirms the healing capacity granted to this plant species in traditional medicine. Indeed, all tested extracts have effective antifungal activity coupled with a fungistatic action on the *in vitro* growth of the three fungal isolates. The most active extract is the extract from the F₁₂ fraction. *Trichophyton mentagrophytes* appears to be the most sensitive fungal germ and *Aspergillus fumigatus* the least sensitive. The sensitivity of *Candida albicans* is intermediate. The anti-infective property granted to *Terminalia catappa* is therefore justified. However, further study must be done to determine the chemical structure of the active ingredient with appropriate methods.

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