GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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# Antifungal activity of chromatographic fractions of hexane extract x 1-2 of *Eucalyptus torelliana (Corymbia torelliana)* on the *in vitro* growth of *candida albicans candida tropicalis and candida glabrata*

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GSC Biological and Pharmaceutical Sciences, 2022, 20(01), 277–283

Publication history: Received on 10 June 2022; revised on 16 July 2022; accepted on 18 July 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.20.1.0293

## Abstract

In developing countries (DC), general mortality is mainly due to infectious diseases which outweigh cardiovascular and degenerative diseases as well as cancers. Among these, opportunistic candidiasis is on the rise among HIV/AIDS patients. AIDS remains the leading cause of death in young adults in developing countries. In view of the growth of infectious diseases, our research team tested the chromatographic fractions of the X1-2 *Eucalyptus torelliana* extract (ornamental plants) on the in vitro growth of *candida albicans, candida tropicalis and candida glabrata*. Among these fractions, fraction F5 showed the best activity on the three germs tested with a CMF value of 62.5 µg/mL for each germ.

**Keywords:** Chromatography; Infectious diseases; Candidiasis; *Eucalyptus torelliana*

## 1. Introduction

Of all microbial infections in humans, mycoses, and particularly systemic mycoses, are still those whose often chronic and sometimes fatal evolution is difficult to eradicate [1]. The appearance of strains resistant to the usual antifungal agents, the emergence of pathogenicity in strains that are usually saprophytic in our environment and the immunodepressions caused by HIV infection are the main factors of this strong recrudescence [2; 3; 4; 5].

To overcome these problems, the search for new drugs from medicinal plants continues for many scientific teams [6; 7; 8]. Our team is part of this logic by having as an approach the exploration of plants of the Ivorian pharmacopoeia in order to identify those that could enter the manufacture of new antimicrobial drugs. Among the phytomedicines under evaluation by our team, extracts of *Terminalia superba, Thonningia sanguinea,* have shown a wide spectrum of antifungal and antibacterial activity [9;10]. However, the study on the tree *Eucalyptus torelliana* or *Corymbia torelliana*, which is a service plant, is insufficient. This study therefore aims to evaluate the anticandidosic activity of some extracts of *Eucalyptus torelliana*, codified EUCA on the in vitro growth of Candida albicans Candida tropicalis and Candida glabrata in Côte d'Ivoire.

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## 2. Material and methods

### 2.1. Plant material

The material used is the powder obtained from the bark of *Eucalyptus torelliana*, codified EUCA. This plant was identified by the National Floristic Center of the University Felix Houphouet-Boigny of Abidjan-Cocody.

### 2.2. Germs tested

The germs used as test in this study were provided by the laboratory of mycology of the Pasteur Institute of Abidjan (Ivory Coast) namely.

*Candida albicans* and *Candida tropicalis* collected from patients with deep mycoses and *Candida glabrata* isolated from patients with superficial mycoses.

### 2.3. Culture medium

For the tests, sabouraud agar (Bio-RAD/Ref: 64449; Lot: 8B2212) at acid pH (5.7) was used.

### 2.4. Methods

#### *2.4.1. Inventory and mapping of Eucalyptus*

The tool used to collect X and Y coordinates of Eucalyptus is a GPS Garmin 64s.

The collection method was based first on the identification of the species studied. Thus, using the GPS, the coordinates of each identified tree were recorded by walking the entire area of the site of interest. In total, 103 trees were observed and collected. The data collected was then transformed into geographic information using mapping software. Within the framework of this work, the Arcgis 10.5 software was used for the cartographic restitution.

#### *2.4.2. Preparation of extracts*

Pieces of bark of *Eucalyptus torelliana* harvested, cut were dried in the shade, then finely crushed using an electric grinder. From the powder obtained, total aqueous and hydroalcoholic extracts were prepared. Thus: One hundred (100) grams of EUCA were extracted by homogenization with a blender in one liter of solvent mixture comprising 70% ethanol and 30% distilled water. After six cycles of grinding, the resulting homogenate was first wrung out in a clean cloth square and then successively filtered twice on absorbent cotton and 3 mm Wattman filter paper. The filtrate was dried in a Büchi type rotary evaporator at a temperature of 40 °C. The powder obtained constitutes the hydroalcoholic extract coded X0. Subsequently, 10 grams of X0 were constituted and subjected to partitioning in 100 mL of a 50/50 hexane-water solvent mixture (v/v). After decantation, the two phases were separated and concentrated using a rotary evaporator. The following extracts were obtained respectively:

X1.1: the hexane phase, X1.2: the aqueous phase from the hexane-water partition.

The extract X1-2 was retained for its optimization in the further work considering that it showed a better activity on the tested germs [11; 12]. Thus a quantity of 3 mg of this extract was taken, diluted with distilled water and then chromatographed on a Sephadex G50 gel filtration column. The eluent used is distilled water. The characteristics of the column are: diameter = 2 cm; gel height =37 cm; flow rate = 0.24 mL/min. One hundred and ten sub-fractions of 10 mL were collected, so using the thin layer chromatographic profile of the sub-fractions of the X1-2 extract, different fractions were constituted (F1, F2, F3, F4, F5, F6, F7, F8, F9, F10). These extracts were then tested separately on the in vitro growth of the 3 *Candida* strains.

#### *2.4.3. Preparation of the culture medium*

The Sabouraud medium was prepared according to the indications of the supplier (BIO-RAD/Ref: 64449; Lot: 8B2212): 42 g of the medium (in powder form) were homogenized in 1000 mL of distilled water (for the manipulation needs, we took the corresponding quantity). This mixture was stirred and heated on a heated magnetic stirrer (IKA-MAG-RCT).

#### *2.4.4. Incorporation of the vegetable extracts*

The incorporation of the different plant extracts to Sabouraud agar was done according to the double dilution method in inclined tubes [10; 13; 14). For each plant extract, each set consists of ten test tubes. Eight of these test tubes contain

the plant extracts. And the other two tubes are considered as control tubes, one of which without plant extract without germs serves as a control for the sterility control of the culture medium and the other without plant extract serves as a control for the growth control of germs. For the eight test tubes the concentrations vary from 500 to 3.906 µg/ml according to a geometric bond of reason ½.

After incorporation of the extracts, all ten tubes of each extract were autoclaved at 121°C for 15 minutes and then tilted with small pellet at laboratory temperature to allow their cooling and solidification of the agar [10; 13; 14]

*2.4.5. Antimicrobial test*

Antimicrobial assays were performed similarly for the three Candida strains tested. From young Candida culture (48 hours of incubation), the inoculum was prepared as follows:

A young *Candida* colony collected with a loop was homogenized in 10 mL of sterilized distilled water. This gave the stock suspension (100) concentrated to 106 cells/mL. From this suspension, a second suspension (10-1) was prepared by dilution to 1/10th of the first. It carries a load of 105 cells/mL [15; 16].

For each of the test tubes in each set of the ten extracts (except the sterility control tube of the culture medium), the sprout culture was made on the previously prepared media by seeding 10 µl corresponding to 1000 cells of the 10-1 suspension in cross streaks until exhaustion. The cultures thus made were incubated at 30°C for 48 hours.

After the incubation time, Candida colonies were counted by direct counting using a colony counter pen (Bel-Art brand Scinceware serial number 23382). Growth in the eight experimental tubes of each series was evaluated as percent survival calculated against 100% survival in the growth control tube [10; 13] Processing of the experimental data allowed the determination of the following antifungal parameters:

- The minimum inhibitory concentration (MIC): this is the minimum concentration for which there is no growth visible to the naked eye.
- Minimum fungal concentration (MFC): this is the lowest concentration of extract in the tube that gives 99.99% inhibition compared to the growth control tube or conversely it is the tube that leaves a 0.01% survival compared to the growth control tube. It is determined by a sterility test of the tube corresponding to the MIC by plating a sample taken from the surface of the agar of this tube on a new agar.
- Concentration for 50% inhibition (IC50): it is the concentration that gives 50% of estimated inhibition compared to the number of colonies counted in the growth control tube. This parameter is determined graphically.

The fungicidal or fungistatic effect was revealed after subculturing the contents of the tube corresponding to the MFC and the cell count was possible thanks to the different dilutions of the 10-1 suspension.

## 3. Results

### 3.1. Classification of the studied species

According to APG IV (Angiosperm Phylogeny Group), 2016 the classification of the studied species is as follows:

- Domain: Biota Endl.(D.Don)
- Kingdom: Plantae Haeckel, 1866
- Sub-Region: Viridaeplantae
- Sub-Region: Streptophyta John, Williamson & Guiry, 2011
- Class: Equisetopsida C.Agardh, 1825
- Clade: Tracheophyta Sinnott ex Cavalier-Smith, 1998
- Clade : Spermatophyta
- Subclass : Magnoliidae Novák ex Takht, 1967
- Super-Order: Rosanae Takht, 1967
- Order: Myrtales Juss. ex Bercht. & J.Presl, 1820
- Family: Myrtaceae Juss., 1789
- Subfamily: Myrtoideae Sweet, 1827
- Tribe : Eucalypteae Peter G.Wilson, 2005

- Genus : Corymbia K.D.Hill & L.A.S.Johnson, 1995
- Species: *Corymbia torelliana* (F.Muell.) K.D.Hill & L.A.S.Johnson
- Hierarchical classification [17]

### *3.2.* Distribution and use of *Corymbia torelliana*

*Eucalyptus* or *Corymbia* are trees used for reforestation. On the site of the university campus of Cocody Abidjan, these trees were used for the ornament of the university. A total of 103 Eucalyptus trees are distributed throughout the area. Stands are observed in the "Palm Tree" area, on the site of the Faculty of Medicine and the Ecole Normale Supérieure (ENS). Isolated trees are observed in the Botanical Garden of the University, at the University Center for Research and Application in Remote Sensing (CURAT) and the Faculty of Odonto-Stomatology. They are mature trees in danger of extinction.

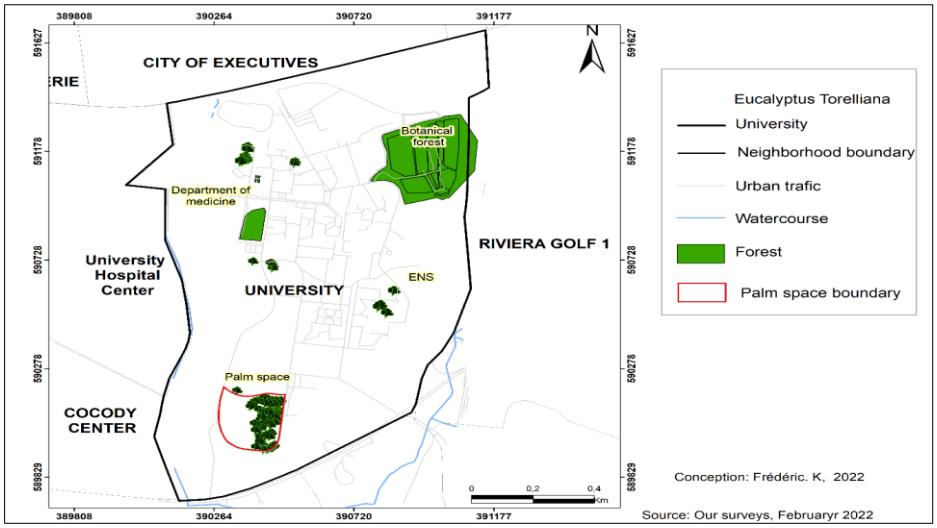


**Figure 1** Distribution of *Corymbia torelliana*

### 3.3. Chromatographic fractions

**Table 1** Comparative antifungal parameter values in µg/mL for the Fractions of extract **$X_{1-2}$**

| | | Fungal germs | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***C. albicans*** | | ***C. glabrata*** | | ***C. tropicalis*** | |
| | | **CMF** | **$CI_{50}$** | **CMF** | **$CI_{50}$** | **CMF** | **$CI_{50}$** |
| $X_{1-2}$ | $X_{1-2}$ | 125 | 21,19 | 125 | 08,62 | 125 | 09,91 |
| G1 | $F_1$ | > 500 | Ind | > 500 | Ind | >500 | Ind |
| | $F_2$ | > 500 | 203,12 | > 500 | 5,85 | > 500 | 93,75 |
| G2 | $F_3$ | 125 | 29,30 | 125 | 13,66 | 125 | 09,23 |
| | $F_4$ | 125 | 14,50 | 125 | 11,71 | 125 | 06,87 |
| G3 | $F_5$ | 62,5 | 06,45 | 62,5 | 01,73 | 62,5 | 02,70 |
| G4 | $F_6$ | 250 | 86,80 | 250 | 19,95 | 250 | 28,63 |
| | $F_7$ | 250 | 117 | 250 | 21,47 | 250 | 41,01 |
| | $F_8$ | 250 | 145,83 | 250 | 26,03 | 250 | 36,45 |
| | $F_9$ | 250 | 55,55 | 250 | 14,75 | 250 | 22,76 |
| | $F_{10}$ | 500 | 164 | 500 | 14,64 | 500 | 21,86 |

Ind : Indeterminate; X1-2 : aqueous phase from the hexane/water partition; F1 : fraction 1, F2 : fraction2, F3 : fraction3, F4 : fraction 4, F5 : fraction 5, F6 : fraction 6; F7 : fraction 7, F8 : fraction 8, F9 : fraction9, F10 : fraction10; G1 : group 1 ; G2 : group 2 ; G3 : group3 ; G4 : group 4

Column chromatography yielded 10 fractions tested separately on each of the Candida germs with a concentration range from 500 to 3.906 µg/mL. The antifungal tests on the in vitro growth of the 3 germs show in the majority of cases a reduction in the number of colonies in the experimental tubes compared to the control tube and this as the concentration of the extract increases in the experimental tubes. The activity of the active fractions is therefore dose-dependent. Thus, some of these fractions (F1 and F2) show no inhibition in the established concentration range, others show a clear and effective inhibitory activity (F3 to F10). The values of the antifungal parameters are recorded in Table 1.

## 4. Discussion

The floristic inventory allowed us to identify 103 Eucalyptus trees. This species is used as a service plant on the university campus of Cocody. It is a fast growing tree and is used as a street and garden tree for ornamentation. In other environments, it is present on reforestation sites because it is a tree that can reach 25 to 30 m in height. All this shows the availability of the species for biochemical studies.

Compared to the CMF values of the basic extract X1-2 and depending on the concentration range, the obtained CMF or IC50 values allow to group the fractions. Thus, we obtain 4 groups of fraction:

- Group G1 which concerns the fractions that have no net and effective inhibitory activity at the maximum value of 500 µg/mL. These are the F1 and F2 fractions.
- Group G2, these are the fractions that inhibit the in vitro growth of the 3 fungal germs and whose CMF values are equal to those of the basic extract X1-2. These are fractions F3 and F4.
- Group G4, these are the fractions that inhibit the in vitro growth of the 3 fungal germs and whose CMF values are higher than those of the basic extract X1-2. These are fractions F6, F7, F8, F9 and F10.
- Group G3 is the fraction that effectively presents a clear and effective inhibitory activity on the in vitro growth of the 3 fungal germs and whose CMF value is lower than that of the basic extract X1-2. This is fraction F5.

From the comparison of the fractions of the group G1 between them on the basis of the IC50 values, it appears that the most active fraction is the fraction F2 although their CMF values are not determined.

On the other hand, concerning the fractions of group G2, the fractions F3 and F4 have the same CMF values. But based on the IC50 values, fraction F4 is the most active on the three fungal germs. Moreover, these two fractions are more active than the fractions of group G1.

With the fractions of group G4, it appears that fractions F6, F7, F8, F9 have the same activities on C. albicans, C. tropicalis and C. glabrata, but F9 is the most active on the basis of IC50. After F9 come F6, F7, and F8 for Candida albicans. On the other hand, for Candida glabrata and Candida tropicalis come after the F9 fraction, the F8, F6 and F7 fractions.

Of this group the lowest activity is obtained with fraction F10. The comparison of the activities of group G4 with those of groups G1 and G2 shows that the activity of the fractions of group G4 is better than that obtained with the fractions of group G1. However, the fractions of group G2 have a higher activity than the fractions of group G4.

Regarding the fraction of group G3 (F5) the analysis reveals that this fraction has the best CMF value compared to the CMF values obtained with the fractions of groups G1, G2 and G4. Furthermore, the comparison on the basis of IC50 values shows that the activity of the F5 fraction varies from germ to germ. Thus, F5 is more active on C. glabrata and C. tropicalis. Of these two germs the activity is better on C. glabrata. However, C. albicans is more resistant to F5 than C. glabrata and C. tropicalis.

In addition, fraction F5 has a 2-fold better activity than the activity of the extract used as a basis for its preparation (extract X1-2) and 4-fold better than the activity of the total extract X0 used to prepare the extract X1-2 Agré et al. [11]. It is also 8 times more active than the aqueous total extract XAq Agré et al.[11]. These results reveal that the chromatographic fractionation resulted in a better concentration of the active ingredients contained in the X1-2 extract. The antifungal activity of the X1-2 extract was therefore improved by 2-fold. The F5 fraction therefore has the best antifungal activity.

These results obtained are better than those of Kporou et al.[18] with the chromatographed extract F8 of *Mitracarpus scaber* (MFC = 780 µg/mL) on the in vitro growth of C. albicans. Indeed, the F5 fraction is 12 times more active than the F8 fraction of *Mitracarpus scaber*. The same is true for the F1 fraction of *Morinda morindoides* obtained by Bagré [19]

on the in vitro growth of C. albicans for which the FMC value is 780 µg/mL. The F5 fraction is also 12 times more active than the F1 fraction of *Morinda morindoides*.

From this analysis, it can be seen that Sephadex G50 gel column chromatography significantly improved the anti-candidus activity of the X1-2 base extract. This method made it possible to obtain the F5 fraction which represents the most active fraction. Indeed, on the in vitro growth of C. albicans C. glabrata and C. tropicalis, the F5 fraction is 2 times more active than the X1-2 extracts.

## 5. Conclusion

- The floristic inventory has identified 103 trees used as street and garden trees.
- This shows the availability of the species for biochemical and pharmacological studies.
- This study also situates us on the real anti-infectious potential of *Eucalyptus torelliana (Corymbia torelliana*). The results of these investigations allowed us to understand that the extracts of this plant have a more or less accentuated antifungal activity on the in vitro growth of *Candida Albicans, Candida tropicalis and Candida glabrata.* The preparation of the extracts by the method of Zirihi and Kra in 2003 followed by a chromatography on column of G50 sephadex gel allowed to improve considerably the anticandidosic activity of the basic extract X1-2. This method allowed to obtain the F5 fraction which is the most active.

## Compliance with ethical standards

### *Acknowledgments*

We would like to thank:

- To the traditional practitioners who revealed to us the benefits of this plant in traditional environment.
- All the members of the pharmacodynamic-biochemical laboratory for their active participation in this work.
- To the national center of floristics of Ivory Coast for the identification of this plant.

### *Disclosure of conflict of interest*

The authors declare that they have no conflicts of interest in this article

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