Available online on 15.06.2022 at http://jddtonline.info



Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Copyright © 2011-2022 The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited



Open Open Access Full Text Article



**Research Article** 

# Evaluation of the antifungal powers of five plant species of the genus *Terminalia* on strains responsible for candidiasis

Gbouhoury Eric-Kévin BOLOU 1\*, Konan YAO 1, Don Josette AGRE 2, Désirée Ruth Constance NGUESSAN 2, Guédé Noel ZIRIHI 2, Allico Joseph DJAMAN 2

<sup>1</sup> National Center of Floristics, Félix HOUPHOUET-BOIGNY University, Côte d'Ivoire.

<sup>2</sup> UFR Biosciences, Félix HOUPHOUET-BOIGNY University, Côte d'Ivoire.

#### Article Info:

#### Abstract

Received 16 April 2022 Reviewed 18 May 2022 Accepted 22 May 2022 Published 15 June 2022

Article History:

Cite this article as:

Bolou GE, Yao K, Agre DJ, Nguessan DRC, Zirihi GN, Djaman AJ, Evaluation of the antifungal powers of five plant species of the genus *Terminalia* on strains responsible for candidiasis Journal of Drug Delivery and Therapeutics. 2022; 12(3-5):73-76

DOI: http://dx.doi.org/10.22270/jddt.v12i3-s.5370

\*Address for Correspondence:

Gbouhoury Eric-Kévin BOLOU, National Center of Floristics, Félix HOUPHOUET-BOIGNY University, Côte d'Ivoire Background: Candidiasis is a fungal disease caused by *Candida albicans*, a yeast that preferentially affects mucous membranes. These diseases are more and more recurrent because of the generalized decline in immunity and the appearance of resistance to classical antifungal drugs. Therefore, fighting against therapeutic failures becomes necessary. The objective of this study is to contribute to the fight against candidiasis through the research of new antifungal molecules. To do so, the hydroalcoholic extracts of *Terminalia catappa, Terminalia glaucescens, Terminalia ivorensis, Terminalia mantaly and Terminalia superba*, five plant species used in traditional medicine against dermatoses, were tested on the in vitro growth of resistant strains of *Candida albicans*. In addition, the polyphenol composition of these plants was carried out in order to evaluate their protective powers.

Methods: The antifungal tests were performed by the solid-state dilution method. While the determination of polyphenols was performed by the photometric method.

Results: The results showed an inhibition of the growth of germs by four plants except *Terminalia catappa*. The minimum inhibitory concentrations (MIC) obtained are as follows: MIC (*T. ivorensis*) =0.25 mg/mL; MIC (*T. glaucescens*) =0.50 mg/mL; MIC (*T. superba*) = 4 mg/ML; MIC (*T. mantaly*) = 2 mg/mL.

Conclusion: This study indicates that *T. ivorensis* is the most active and has the highest content of phenolic compounds. This plant could be a source of effective molecules in the treatment of candidiasis.

Keywords: Candida albicans, candidiasis, Terminalia, antifungal, polyphenol,

# **INTRODUCTION**

Candidiasis is one of the most frequent fungal pathologies in men. They are caused by Candida albicans, a commensal yeast that preferentially affects the vaginal mucosa <sup>1,2</sup>, making vulvovaginal candidiasis the second most common condition after bacterial vaginosis. An estimated 75% of women will have at least one episode of Candida vaginitis in their lifetime 3,1. In Côte d'Ivoire, Candida albicans accounts for 72.6% of yeast strains isolated from vaginal swabs 4. However, candidiasis is also often found in the mouth and intestinal walls. Conventional treatment of these conditions is based on Amphotericin B, 5-fluorocytosine, Fluconazole, Itraconazole and Voriconazole. But also, the use of plants in traditional medicine has sometimes shown spectacular results in the treatment of various fungal diseases, especially plants belonging to the genus Terminalia. Thus, studies have shown that several species of Terminalia are endowed with antifungal powers <sup>5</sup> including *Terminalia ivorensis* which is very active on Aspergillus fumigatus 6 and Terminalia mantaly, active on Candida albicans 7 . The inappropriate use of classical antifungal drugs has favored the appearance of resistant strains <sup>4</sup> which are responsible for therapeutic failure in the certain candidiasis, management of especially in

immunocompromised individuals. Faced with this difficulty, research of new antifungal molecules effective against candidiasis resistant to classical molecules is necessary. The study of Terminalia species seems to be a promising avenue for the discovery of effective drugs. The general objective of this study is to identify a species of the genus Terminalia likely to contain an active principle effective against candidiasis. To achieve this, the hydroalcoholic extracts of five species of Terminalia, namely: Terminalia catappa, Terminalia glaucescens, Terminalia ivorensis, Terminalia mantaly and Terminalia superba, were prepared, and then, their antifungal powers on C. albicans and antioxidants were evaluated.

# **MATERIALS AND METHODS**

## 1. Material

# **Plant material**

The plant material consisted of the bark of five Terminalia species listed below (Table 1). These plants were collected in Taabo (160 km from Abidjan) and in Agboville (82 km from Abidjan). They were then identified at the Centre National de Floristique of Félix HOUPHOUET-BOIGNY University.

Table 1: Plant material

Plant species	Herbarium numbers	Place of collection
Terminalia catappa	UCJ003136	Agboville
Terminalia glaucescens	UCJ003137	Taabo
Terminalia ivorensis	UCJ0033156	Agboville
Terminalia mantaly	UCJ003173	Agboville
Terminalia superba	UCJ003188	Agboville

#### **Microorganisms**

The germs tested were composed of three strains of *Candida albicans* of oral origin with the following resistance profiles

Candida albicans 2 (susceptible)

*Candida albicans* 370 (Amphotericin B<sup>R</sup>)

*Candida albicans* 479 (Fluconazole<sup>R</sup> Itraconazole<sup>R</sup> Voriconazole<sup>R</sup>)

#### 2. Methods

#### 2.1. Preparation of hydroalcoholic plant extracts

Harvested barks were cleaned and washed with clean water to remove traces of mold and other waste. They were then cut into small fragments and dried in an oven at 50°C for 5 days. These dried barks were then reduced to very fine powders which will be used for the preparation of hydroethanol extracts according to the method described by **Ouattara** *et al.*<sup>8</sup> with some modifications. Indeed, 100 g of plant powder were shaken vigorously in 1L of 70% ethanol using an electric mixer. The resulting mixture was filtered through a sieve and then the residues were mixed in 500 mL of 70% ethanol, twice in a row. Another filtration is performed, once on percale cloth and twice on increasingly tightly packed hydrophilic cotton, in a funnel. The resulting liquid hydroalcoholic extract is evaporated in an oven at 50°C to obtain a dry extract.

#### Calculation of the extraction yield

The extraction yield (ER) is defined as the ratio between the mass of dry hydroalcoholic extract obtained (M') and the mass of plant material (bark powder) used (M). The yield is expressed as a percentage. It is calculated by the following formula:

RE (%) = 
$$\frac{M'}{M}$$
 X100

RE: Extraction yield in %.

M': Mass of dry hydroalcoholic extract obtained in grams. M : Mass of fresh plant material (leaf) used in gram.

# 2.2. Evaluation of antifungal activities of plant extracts

The antifungal activity is evaluated by the solid-state dilution method and the antifungal parameters of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) are determined 9.

# Incorporation of plant extracts into Sabouraud agar

The incorporation of plant extracts into the agar was performed using the double dilution tube method. Each series consisted of 10 tubes containing the plant extract incorporated into the culture medium and 2 control tubes containing gel medium, one of which was without plant extract for germ growth control (TC) and the other without plant extract or germ for sterility control (TS) of the culture medium

#### Journal of Drug Delivery & Therapeutics. 2022; 12(3-S):73-76

<sup>10,11</sup>. The test tubes contain a range of decreasing concentrations of extract that varies from 8 mg/mL to 0.0156 mg/mL by describing a geometric sequence of reason 1/2. To perform the double dilution, 320 mg of plant extract was homogenized in 40 mL of liquefied Sabouraud agar in tube T1, giving a concentration of 8 mg/ mL. The other nine tubes contained 20 mL of liquid agar. Then, 20 mL of the homogeneous mixture from tube T1 was transferred to the next tube (T2), containing 20 mL of Sabouraud agar and this mixture was homogenized. This operation was repeated successively for the other tubes until tube 10 (T10), containing the lowest concentration (0.0156 mg/mL). For this last tube, half of the content was discarded to adjust the volumes. The tubes thus prepared were sterilized at 121 °C by autoclaving for 15 min. The sterile contents of each tube were inverted into a petri dish under sterile conditions and then left at laboratory temperature to allow cooling and solidification of the agar <sup>12</sup>.

#### Fungal inoculum preparation, plating and incubation

The fungal inoculum was prepared from 24 h young colonies. For each strain, a perfectly isolated colony of C. albicans was suspended in 10 mL of sterile distilled water to give a microbial suspension estimated at 106 cfu/mL. A 1:10 dilution of this bacterial suspension was then performed to give our fungal inoculum estimated at 105 cfu/mL. Next, each of the petri dishes containing the extract concentrations were seeded by 5 mm streaks with 10  $\mu$ L of each inoculum. The inoculated petri dishes were incubated at 30°C for 48 h <sup>13</sup>.

#### Determination of antifungal parameters (MIC and MFC)

After 48 h of incubation, the growth of germs is observed in the petri dishes of each series of culture. The Minimum Inhibitory Concentration (MIC) of each C. albicans strain is determined for each extract. This is the minimum concentration for which there is no visible growth of the germ with the naked eye. The Minimum Fungicidal Concentration (MFC) is also determined. This is the lowest concentration of extract that results in at least 99.99% inhibition of germ growth, compared to the growth control. In practice, the MFC was determined by sterility testing of streaks that showed no visible growth from the MIC. Transplants of streaks with no visible growth were performed on a Petri dish containing new Sabouraud agar and containing neither plant extract nor antifungal substance. Indeed, the platinum loop was passed over the surface of each streak without visible microbial growth and then inoculations were made, again per streak, on new agar. After 48 hours of incubation at 30°C, the CMF was identified.

#### 2.3. Determination of phenolic compounds

One milliliter of a methanolic solution of plant extract of concentration 1 mg/mL is introduced into a test tube. 1 mL of Folin-Ciocalteu reagent is added. This tube is then left to stand for 3 min and 1 mL of 20% (w/v) sodium carbonate solution is added. The contents of the tube are made up to 10 mL with distilled water and the tube is placed in the dark for 60 min. Optical Density (OD) reading is then taken at 745 nm against a blank with a spectrophotometer (DRAWELL DU-8200). A calibration line established from a concentration range of a stock solution of gallic acid, at 1 mg/mL, treated under the same conditions as the assay was used to determine the amount of phenolic compound in the sample 14,15.

# **RESULT AND DISCUSSION**

The preparation of hydroethanol extracts from 100 g of plant powder yielded 10.13 g of *T* superba extract, 13.34 g of *T* glaucescens, 21.64 g of *T* ivorensis, 10.47 g of *T* mantaly and 12.63 g of *T* catappa, corresponding to 10.13%, 13.34%, 21.64%, 10.47%, and 12.63%, respectively (Figure 1). The analysis of this result shows that *Terminalia ivorensis* has the best extraction efficiency. This is in agreement with the work of **Moomin et al** <sup>16</sup> who obtained such high yield with the same plant. This result means that this plant is relatively more concentrated in secondary metabolites extractable by ethanolwater solvent (70/30, v/v).

The study of in vitro antifungal potencies on candida albicans gave the results reported in Table 2 below. These results show that *Terminalia ivorensis* has the highest inhibitory power with an MIC of 0.25 mg/mL and a BMC of 2 mg/mL on each of the three strains of *candida albicans*. Next comes *Terminalia glaucescens* which inhibits each of the strains with an MIC of 0.5 mg/mL. On the other hand, *Terminalia catappa* showed no inhibition of the fungal strains up to the maximum concentration used. Its MIC is certainly higher than 8 mg/mL. This plant is the least active on C. albicans strains, among the five species studied.

#### Table 2: Antifungal parameters of different Terminalia species

#### Journal of Drug Delivery & Therapeutics. 2022; 12(3-S):73-76



Figure 1: Extraction yields

	FUNGAL STRAINS						
Plant extracts	Candida a	Candida albicans 2 Candida albicans 370		lbicans 370	Candida albicans 479		
	MIC	MFC	MIC	MFC	MIC	MFC	
Terminalia ivorensis	0,25	2	0,25	2	0,25	2	
Terminalia superba	4	8	4	8	4	8	
Terminalia glaucescens	0,5	2	0,5	2	0,5	2	
Terminalia Mantaly	4	>8	2	8	2	8	
Terminalia catappa	>8	>8	>8	>8	>8	>8	

This study also shows that the hydroethanolic extracts of the four plants *T* superba, *T* glaucescens, *T* ivorensis and *T* mantaly are active, with a predominance of T. ivorensis, on Candida albicans strains resistant to Amphotericin B, Fluconazole, Itraconazole and Voriconazole, which are reference molecules in the treatment of candidiasis. The results show that these plants contain active ingredients that are effective on these resistant strains and that *T. ivorensis* is more concentrated in anti-candidus active ingredients. This observation is in phase with the extraction yields obtained. The active ingredients of T. ivorensis could substitute the classical molecules and cure the cases of resistant candidiasis. Previous works have shown that Terminalia are generally endowed with antifungal powers <sup>17,18</sup> and *T. ivorensis* is particularly very active, notably on Aspergillus fumigatus 6 and Candida albicans. Therefore, this plant can be used in the composition of a biopesticide and a

medical cream for its antifungal activity <sup>19,20</sup>. The study of the polyphenol content of these five plants shows that their compositions differ from each other. Indeed *T. ivorensis* is the richest in polyphenols with 93.76 mg EAG/g. *Terminalia glaucescens* comes second with 80.79 mg GAE/g. Then follow respectively *T. catappa* 68.35 mg EAG/g and *T. mantaly* 62.49 mg EAG/g. *Terminalia superba* is the least rich in phenolic compounds with 20.79 mg EAG/g (Figure 2). The high content of phenolic compounds in *T. ivorensis* indicates that this plant might be endowed with antioxidant property <sup>21</sup>. This property would be useful in the protection and repair of mucosal tissues infected by *Candida albicans*.

Finally, among the five Terminalia species studied, Terminalia ivorensis appears to be the best candidate for the preparation of phytomedicines or extraction of molecules effective against resistant candidiasis.



Figure 2: Total polyphenol content of hydroethanol extracts of Terminalia

#### Bolou et al

# **CONCLUSION**

This study, which evaluated the inhibition potential of five *Terminalia* species on resistant and multi-resistant strains of *Candida albicans*, revealed that *Terminalia ivorensis* is a promising plant. It could be used for the preparation of improved phytomedicines or for the extraction of new antifungal molecules capable of effectively curing resistant candidiasis. This study also constitutes a justification for the use of plants of the genus *Terminalia* in the traditional treatment of mycosis in general.

# **CONFLICT OF INTEREST**

There are no conflicts of interest declared by the authors

## **REFERENCES**

- 1. Bergogne-Bérézin E. Flores vaginales normales, vaginites et vaginoses bactériennes: diagnostic et thérapeutique. Antibiotiques, 2007; 9 :139-44. Doi : ANTI-05-2007-9-2-1294-5501-101019-200701945
- Sobel JD. vulvovaginal candidosis. Lancet, 2007; 369:1961-71. DOI: https://doi.org/10.1016/S0140-6736(07)60917-9
- Pihet M. et Marot A. Diagnostic biologique des candidoses. Revue Francophone des Laboratoires, 2013; 450:47–61. DOI: https://doi.org/10.1016/S1773-035X(13)71946-4
- Djohan V., Angora K.E., Vanga-Bosson A.H., Konaté A., Kassi F.K., Yavo W., Kiki-Barro P.C., Menan H., Koné M. Sensibilité in vitro des souches de Candida albicans d'origine vaginale aux antifongiques à Abidjan (Côte d'Ivoire), Journal de Mycologie Médicale, 2012; 22(2):129-133. DOI: https://doi.org/10.1016/j.mycmed.2011.11.005
- Batawila K. Diversité, écologie et propriétés antifongiques des Combretaceae du Togo, Acta Botanica Gallica, 2002; 149 (4):515-516. DOI: https://doi.org/10.1080/12538078.2002.10515979
- Ouattara S., Kra K.A.M., Kporou E. et Guede-Guina F., Évaluation de l'activité antifongique des extraits de *Terminalia* ivorensis (tekam 2) sur la croissance *in vitro* de *aspergillus fumigatus*. Bulletin de la société royale des sciences de liège, *volume 2009; 78:302 – 310.* DOI : https://popups.uliege.be/0037-9565/index.php?id=1590.
- Yayé Y.G., Kra A.K.M., Ackah J.A.A.B. & Djaman A.J. Evaluation de l'activité antifongique et essai de purification des principes actifs des extraits de *Terminalia mantaly* (h.perrier), une combretacée, sur la croissance *in vitro* DE *Candida albicans.»*, *Bulletin de la Société Royale des Sciences de Liège*, 2011; 80:953 – 964. DOI : https://popups.uliege.be/0037-9565/index.php?id=3480.
- 8. Ouattara Abou, Bolou Gbouhoury Eric-Kévin , Koffi Allali Eugène, Yasseu Hyacinthe, Ouattara Karamoko and Coulibaly Adama. Antifungal activity of *Pericopsis* (Afrormosia) *laxiflora* (Benth.) Bark on ringworm germs. Microbiology Research Journal International, 2020; 30(4):1-9. DOI: https://doi.org/10.9734/MRJI/2020/v30i430207
- Fezan HT., N'guessan FK., Favel A. & Fallague K. Activité antifongique de quelques plantes de la flore ivoirienne. *Sciences et Nature*, 2007; 4(2):117-122. DOI: https://doi.org/10.4314/scinat.v4i2.42136
- 10. Ahon Gnamien Marcel. Evaluation et essai d'optimisation de l'activite antifongique des extraits de Terminalia superba Engl &

Diels (Combretaceae) SUR LA CROISSANCE in vitro DE Aspergillus fumigatus, Candida albicans et Cryptococcus neoformans, thèse de doctorat unique, université FHB, Côte d'ivoire, 2014, 117 pages.

- 11. Thès PM. Recherche du profil antimicrobien des huiles de G243 et MISCA sur quelques agents de mycose de la peau, Mémoire de DEA de biotechnologies, option pharmacologie microbiologie. Université de Cocody, Côte-d'Ivoire, 2001 ; 34p.
- Bagré, I., Bahi, C., Ouattara K., Zirihi GN., Djaman AJ., Coulibaly A., N'Guessan JD. Étude botanique et exploration de l'activité antifongique de Morinda morindoides (Baker) Milne-Redh. sur la croissance in vitro de Cryptococcus neoformans. Phytothérapie, 2011; 9:136-141. DOI: https://doi.org/10.1007/s10298-011-0612-y
- 13. Kra A. K. M. (2001). Evaluation et amélioration par séquençage chromatographique d'une action fongique de MISCA contre Aspergillus fimugatus, Thèse 3<sup>e</sup> cycle, Université de Cocody. Abidjan, 2001, 126p.
- 14. Djamilatou Z.S., Djibo A.K. Sahabi B. & Seini S.H. (2021). Screening phytochimique, dosage des polyphénols et détermination de l'activité antioxydante de deux plantes anti-hypertensives du Niger. European Scientific Journal, 2021; 17(17):335. DOI: https://doi.org/10.19044/esj.2021.v17n17p335
- Kouamé T.K, Sorho S., Kassi A.B.B. et Yaya Soro Y. Détermination des teneurs en polyphénols totaux, flavonoïdes totaux et tanins de jeunes feuilles non encore ouvertes de Piliostigma thonningii (Caesalpiniaceae). Int. J. Biol. Chem. Sci. 2021; 15(1):97-105. DOI : https://doi.org/10.4314/ijbcs.v15i1.9
- Moomin, A., Mensah, K.B., Forkuo, A., Adu-Gyamfi, P.K.T. And Ansah, C. Ethanolic stem bark extract of terminalia ivorensis A. Chev. protects against potassium dichromate-induced nephrotoxicity in rats. Scientific African, 2020; 8, 10p. DOI: https://doi.org/10.1016/j.sciaf.2020.e00410
- 17. Angaman R.K., Abo K., Orsot B.M.A.B., Ouattara D., Zirihi N.G. Evaluation de l'activité antifongique in vitro et in vivo d'extraits de Terminalia ivorensis et Terminalia superba sur Fusarium oxysporum. Journal Of Humanities And Social Science, 2020; 25(3):11-18. DOI: https://doi.org/10.9790/0837-2503081118
- Soumahoro I.A., D'almeida K.M.A, Gnahoue G., Kra A.K.M., Djaman A.J. evaluation of ointment activity based on terminalia mantaly extract. journal of drug delivery & therapeutics. 2016; 6(5):41-45. DOI: http://dx.doi.org/10.22270/jddt.v6i5.1314
- 19. Angaman R.K., Abo K., Ouattara D., Zirihi N.G. Formulation d'un biofongicide à partir d'extraits de Terminalia superba et Terminalia ivorensis et évaluation de son activité antifongique in vitro et in vivo sur phytophtora capsici et Fusarium oxysporum. Journal of Applied Biosciences, 2020; 146:15005-15015. DOI: https://doi.org/10.35759/JABs.v146.3
- Ouattara S., N'Guessan C. D.R, Bagré I, Kporou K. E., Kra A.K.M. Djaman A.J. (2020). Formulation of an Ointment from Hydroethanolic Extract of Terminalia ivorensis and Therapeutic Trial on Volunteers with Moths. J. Nat. Prod. Resour, 2020; 6 (2):262–264. DOI: https://doi.org/10.30799/jnpr.093.20060202
- Awa, D., Konan, Y., Youssouf, S., Honora, T. B. F., Adama, B., & Witabouna, K. M. Pouvoir antioxydant et teneurs en composés phénoliques de deux espèces du genre Albertisia: Albertisia cordifolia (Mangenot & J. Miège) Forman et Albertisia scandens (Mangenot & J. Miège) Forman (Menispermaceae). European Scientific Journal, ESJ, 2018; 14(30):128. Doi: https://doi.org/10.19044/esj.2018.v14n30p128