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Evaluation of the Antifungal Activity of Five Medicinal Plants on the *In vitro* Growth of a Multi-resistant Strain of C*andida albicans*

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Abstract

This paper focuses on determining the antifungal potential of plants traditionally used to treat infections. Five (5) plants were collected. The anti-*Candida albicans* activity was evaluated by the double dilution method in tilted tubes with the aqueous and hydroethanolic extracts of the different plants. The results showed that 70% ethanolic extracts were more active than the aqueous extracts for all the plants. The 70% ethanolic extract of *Terminalia ivorensis* (MFC = 1.56 mg/mL) was the most active on the tested strain. This was followed by *Terminalia Superba* (MFC = 3.125 mg/mL), *Distemonanthus benthamianus* (MFC = 50 mg/mL), *Gmelina arborea* (MFC= 50 mg/mL), and *Justicia secunda* (MFC > 50 mg/mL). The phytochemical screening revealed the presence of seven (7) major groups of compounds. Saponosides are present only in the aqueous extracts of plants. Polyphenols, Flavonoids, Polyterpenes,

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and Sterols as well as Tanins are present in both alcoholic and aqueous extracts. These compounds could justify its activity and its traditional use. The interesting results obtained with the hydroethanolic extract of *Terminalia ivorensis* incite further research with this extract. A bio-guided study on the fractions from this extract could lead to the discovery of one or more molecules of interest in the fight against pathogenic fungi.

Keywords: Phytochemical screening, Antifungal activity, Medicinal plants, *Candida albicans*

Introduction

Fungal infections have increased dramatically in recent years and have become a major public health concern (Nivoix *et al.*, 2018). The *Candida* genus includes commensal microorganisms, which cause the majority of opportunistic infections (Develoux & Bretagne 2005; Kettani *et al*., 2006). Their pathogenicity is particularly evident in the presence of favorable factors, such as the immunodeficiency of patients (Ascioglu *et al.*, 2002) and the development of drug resistance in certain strains (Granier, 2003). In addition, the cytotoxicity of systemic antifungal agents is another problem encountered in the control of fungal infections (Lin *et al.*, 2001). This has led to the search for new antifungal agents of natural origin. Following the example of certain micro-organisms such as the genera *Penicillium* or *Streptomyces* which have been identified as the source of antimicrobials, certain plants have been used for several centuries by traditional medicine to fight infections. These medicinal plants are mostly used in some Asian and African countries by the population to treat various health problems (Ackah, 2004; Ouattara *et al.*, 2013). Therefore, this study was oriented towards these plants to contribute to the search for new antimicrobials. Among the numerous plant species identified in Ivory Coast, five plants have been selected (Kra *et al.*, 2014). Aqueous decoctions or macerations (aqueous and alcoholic) of the different organs of these plants are used in Ivory Coast and other countries in sub-Saharan Africa to fight diarrhea and skin infections (Adjanohoun & Aké Assi, 1979). The aim of this study is to evaluate the antifungal power of two extracts from each plant on a multi-resistant strain of *Candida albicans.*

Materials and Methods

Plant Material

The various plant organs were harvested in April 2021 in southern and eastern Ivory Coast. Thereafter, they were sorted, washed, cut into small pieces, and dried away from the sun for 21 days before being ground into fine powder.

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Table 1. Organ taken from each plant

Plants	Organ removed	Harvest area
Distemonanthus benthamianus	Trunk bark	Abengourou
Gmelina arborea	Trunk bark	Abidjan
Justicia secunda	Leafy twigs	Abidjan
Terminalia ivorensis	Trunk bark	Agboville
Terùinalia superba	Trunk bark	Agboville

Fungal Material

The anti-candidus activity was performed on *Candida albicans* strain number 479. This multi-resistant strain was provided by the Institut Pasteur of Côte d'Ivoire.

Table 2. Strain tested

Strain	Profile	Culture medium	Incubation temperature	Incubation time
Candida albicans	FCA^R, IT^R, VRC^R	Sabouraud without chloramphenicol	30°C	48h

FCA^R: $fluconazole^R$, IT^R : $itraconazole^R$, VRC^R : $voriconazole^R$

Plant Extraction

The different parts of the plants were harvested, cut, and dried away from the sun for 21 days. After drying, the organs were finely ground separately using an electric grinder. The powder obtained was used for the different extractions. The aqueous and ethanolic extracts were prepared as follows: one hundred (100) grams of powder were extracted by homogenisation in one liter of distilled water in a blender. After six grinding cycles, the homogenate obtained in each case was first wrung out in a clean white cloth square and then successively filtered twice on cotton wool and on Whatman 3 mm filter paper. The filtrate obtained was dried in a Venticell oven. The powder of variable color obtained from one plant to another constitutes the total aqueous extract. The hydroalcoholic extract was prepared by the same process using a solvent mixture of 70% ethanol and 30% distilled water. The yield of each extraction was carried out and expressed as a percentage (Kra *et al.*, 2014).

Phytochemical Screening

The different chemical groups were characterised with reference to the techniques described in the work of Békro *et al*. (2007).

Sterols and polyterpenes were sought by the Liebermann reaction. Five (5) mL of each of the two extracts (aqueous and 70% ethanolic) were evaporated on a sand bath. The residue was dissolved in 1 mL of acetic anhydride while hot and 0.5 mL of concentrated sulphuric acid was added to the triturate. The appearance of a purple or violet ring at interphase, turning blue and then green, indicated a positive reaction.

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The reaction with ferric chloride ($FeCl_3$) was used to characterise polyphenols. A drop of 2% alcoholic ferric chloride solution was also added to two (2) mL of each extract (aqueous and 70% ethanol). The appearance of a more or less dark blue-black or green coloration was the sign of the presence of polyphenols.

Flavonoids were determined by the cyanidin reaction. Two (2) mL of each extract was evaporated and the residue was taken up in 5 mL of 2-fold diluted hydrochloric alcohol. The addition of 2-3 magnesium chips spurred a release of heat and a pinkish-orange or purplish coloration. The addition of 3 drops of isoamyl alcohol intensified this coloration, which confirmed the presence of flavonoids.

Catechic tanins were tested using Stiasny's reagent. Five (5) mL of each extract was evaporated to dryness. After adding 15 mL of Stiasny's reagent to the residue, the mixture was kept in a water bath at 80°C for 30 minutes. The observation of a coarse flake precipitate characterised the catechic tannins. For the gallic tannins, the previous solution was filtered. The filtrate was collected and saturated with sodium acetate. The addition of 3 drops of $FeCl_3$ caused the appearance of an intense blue-black coloration, which is a sign of the presence of gallic tannins.

The quinone substances were determined using Bornstraëgen's reagent. Two (2) mL of each of the two extracts were evaporated to dryness. The residue was triturated in 5 mL of 1/5 hydrochloric acid. The triturate was poured into a test tube and heated in a water bath for 30 minutes. After cooling, it was extracted with 20 mL of chloroform. Ammonia diluted 2 times (0.5 mL) was added to the chloroform solution. A red or purple coloration was the sign of the presence of quinones.

Alkaloids were characterised using Burchard (iodine-iodide reagent) and Dragendorff (potassium iodo-bismuthate reagent) reagents. Six (6) mL of each solution was evaporated to dryness. The residue was taken up with 6 mL of 60° alcohol. The addition of 2 drops of Dragendorff's reagent to the alcohol solution caused a precipitate or an orange coloration. Subsequently, the addition of 2 drops of Burchard's reagent to the alcoholic solution produced a reddish-brown precipitate and indicated a positive reaction.

To test for saponosides, 10 mL of each aqueous extract was poured into a test tube. The tube was shaken for 15 seconds and then left to stand for 15 min. A persistent foam height of more than 1 cm indicated the presence of saponosides.

Antifungal Test

The antifungal activities were assessed by determining antifungal parameters values which are MFC (minimal fungicidal concentration; concentration that inhibit 99.99% of growth in the experimental tube

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compared to the witness tube of growth control) and IC_{50} (Concentration for 50% of inhibition; graphically determined) around each assay. The antifungal tests were carried out on culture medium Sabouraud without chloramphénicol. The inoculum was prepared from a young culture of *Candida albicans* (48 hours). This colony of *Candida albicans* was collected with a loop and homogenized in 10 mL of sterilized distilled water. Thereafter, the mother suspension (10^0) was concentrated to 10^6 cells/mL. From this suspension, a second suspension (10^{-1}) was prepared by dilution to 1/10th of the first, which carries a load of 10^5 cells/ml.

The incorporation of the different plant extracts into Sabouraud agar was done using the double dilution method in slant tubes. For each plant extract, each series consists of ten test tubes. Eight of these test tubes contain the plant extracts, while the other two tubes are considered as control tubes. The one without plant extract is used as a control for the sterility control of the culture medium and the other without plant extract is used as a control for the growth control of germs. For the eight test tubes, the concentrations vary from 100 to 0.78 mg/mL by double dilution according to a geometric bond of reason ½.

After incorporation of the extracts into the eight test tubes, all ten tubes in each series were autoclaved at 121°C for 15 minutes and then tilted with small pellets at laboratory temperature to allow cooling and solidification of the agar.

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

After 48 hours of incubation, *Candida albicans* colonies were counted by direct counting using a colony counter pen. Growth in the eight experimental tubes of each series was evaluated as percent survival calculated against 100% survival in the growth control tube (Ouattara *et al.*, 2013).

Result

Extraction Efficiency

After drying the different extracts, the extraction yields were calculated and recorded in Figure 1. The results show 22.32 ±1.33 and 16.5 ±2.33 for *Distemonanthus benthamianus*, 15.29 ±1.33 and 9.67 ±1 for *Gmelina arborea*, 27.9 ±1 and 19.15 ±1 for *Justicia secunda*, 28.29 ±1.14 and 19.48 ±1 for *Terminalia ivorensis*, and 32.58 ±0.12 and 22.56 ±2.1 for *Terminalia superba*. The aqueous extracts of the different plants recorded the best yields compared to the hydroethanol extract. *T. superba* was the plant

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with the best yields with both the aqueous extract and the 70% ethanolic extract. *Gmelina arborea* recorded the lowest yield.

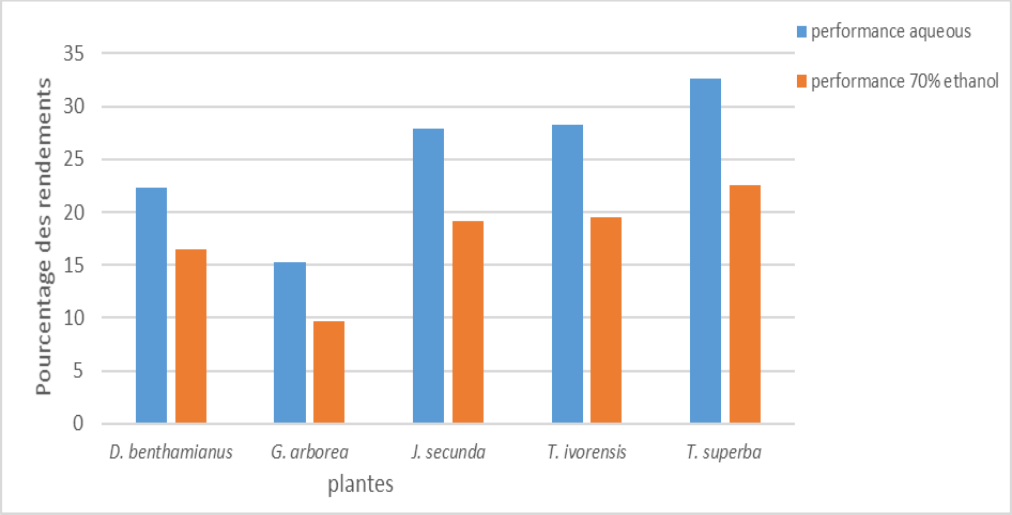

Figure 1. Extraction performance

Qualitative Assay

The phytochemical study of the different extracts gave the results presented in Table 3. The hydroethanolic extracts did not contain saponosides. The absence of tannins and quinones was observed in the *Justicia secunda* extracts. The extracts of *Distemonanthus benthamianus* did not contain catechic tannin and quinones. Polyterpenes, phenolic compounds, and flavonoids were present in all extracts of the different plants. These results reveal that the secondary metabolite composition is variable from one extract to another for the same plant. These variabilities are also seen from plant to plant for the same extract.

Table 3. Phytochemical screening

		Chemical groups								
Plants	Extracts	Sterols and	Polyphenol	Flavonoids	Tanins		Quinones	Alkaloids		Saponoside
					Gallics	Catechic		burchard	dragendorff	
D. benthamianus	Aqueous	+	+	+	+	-	-	+	-	+
	70% ethanol	+	+	+	+	-	-	+	-	-
J. secunda	Aqueous	+	+	+	-	-	-	+	+	+
	70% ethanol	+	+	+	-	+	-	+	+	-
G. arborea	aqueous	+	+	+	+	+	+	+	+	+
	70% ethanol	+	+	+	+	+	+	+	+	-

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T. ivorensis	aqueous	+	+	+	+	+	+	+	+	+
	70% ethanol	+	+	+	+	+	+	+	+	-
T. superba	aqueous	+	+	+	+	+	+	+	+	+
	70% ethanol	+	+	+	+	+	+	+	+	-

-: Absent, +: Present;

Antifungal Activity

Through the anti-candidus activity, the IC_{50} and the MFC of each plant extract was determined. The antifungal parameters of the different plants are shown in Table 4. The analysis of these results reveals that the IC_{50} range from 1 to 5.2 mg/mL for the aqueous extracts and 0.24 to 2.8 mg/mL for the 70% ethanolic extracts. The least active extract is the aqueous extract of *Distemonanthus benthamianus* (IC_{50} =5.2 mg/mL; MFC>50 mg/mL) and the most active extract is the hydroethanolic extract of *Terminalia ivorensis* (IC_{50} =0.24 mg/mL; MFC=1.56 mg/mL).

Table 4. Antifungal parameters of plant extracts

Plants		***Candida albicans***	
		Antifungal parameters in mg/mL	
		IC_{50}	MFC
D. benthamianus	Aqueous	5.2	>50
	Ethanol 70%.	2.8	50
G. arborea	Aqueous	3.2	>50
	Ethanol 70%.	1.41	50
J. secunda	Aqueous	3.2	>50
	Ethanol 70%.	1.4	>50
T. ivorensis	Aqueous	0.3	3.125
	Ethanol 70%.	0.24	1.56
T. superba	Aqueous	1	25
	Ethanol 70%.	0.54	3.125

Discussion

The antifungal properties of five plants used in traditional medicine was studied. Extractions with distilled water and 70% ethanol were performed on the different organs taken from each plant. Chemical screening of the different extracts using different reagents with proven specificities revealed the presence or absence of the seven secondary metabolites. The secondary metabolites of medicinal plants are the material basis of their clinically curative effects and are important indicators to evaluate the quality of medicinal materials. This has prompted the need to identify them in the extracts obtained.

Sterols, polyterpenes, flavonoids, and phenolic compounds were present in all ten plant extracts. The seven metabolites were found in the

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extracts of *Gmelina arborea, Terminalia ivorensis, and Terminalia superba*. At this stage of the study, the biological activities traditionally attributed to these plants would find their origins in the secondary metabolite composition for each plant. Compounds with antimicrobial potential such as terpenes, phenolic compounds, tannins, and alkaloids were found in all ten (10) plant extracts (Oussou *et al.,* 2004; Bagre *et al*., 2011; Basli *et al*., 2012).

The evaluation of antifungal activity by the double dilution tilt tube method determined the antifungal parameters of each plant extract.

From the comparison of the antifungal parameters of the total extracts of each plant, it appears that the hydroethanolic extract is more active than the aqueous extract. It would therefore concentrate the active antimicrobial principles better. This conclusion is consolidated by the work of Kra *et al*. (2015).

The analysis of the different parameters obtained with the 70% ethanolic extract indicates that *Terminalia ivorensis* (IC_{50} =0.24 mg/mL; MFC=1.56 mg/mL) is the most active plant on the multidrug resistant strain tested. This was followed by *Terminalia superba* (IC_{50} =0.54 mg/mL; MFC=3.125 mg/mL), *Gmelina arborea* (IC_{50}=1.41mg/mL; MFC=50 mg/mL), *Distemonanthus benthamianus* (IC_{50} =2.8 mg/mL; MFC=50 mg/mL), and *Justicia secunda* (IC_{50} =1.4 mg/mL; MFC>50 mg/mL).

Although these plants contain the same groups of secondary metabolites, differences in performance were observed in their antifungal activity. These differences originate from the content of active metabolite in each extract. More so, the structural conformation of the molecules and the arrangement of the chemical functions responsible for the biological activities on each basic nucleus of the secondary metabolites found could also provide an explanation for this difference in result.

When the performance of the hydroethanolic extract of *T. ivorensis* is compared with the hydroalcoholic extracts from other works, it appears that the extracts of *T. ivorensis* have the best antifungal activities. Hence, the analysis reveals that the hydroethanolic extract of *T. ivorensis* (MFC=1.56 mg/mL) is 16 times more active than the 70% ethanolic extract of *Morinda morindoides* (MFC=25mg/mL) (Mandić *et al*., 2009), 256 times more active than the extract of *Entandrophramgma cylindrium* (MFC= 800mg/mL), and 64 times more active than the hydroalcoholic extract of *Khaya ivorensis* (MFC= 100mg/mL) (Kra *et al.,* 2014).

Therefore, it is obvious that the plants of the traditional African pharmacopoeia contributes to the discovery of new effective and accessible antifungal drugs. However, more attention should be given on purifying an active principle rather than using the plant itself or its total extract. This is because, very often, plants contain several active principles and constitute

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natural therapeutic combinations, which could exert a synergistic action and prevent the development of resistance in microorganisms.

Conclusion

Five plants used in traditional medicine for microbial diseases were employed in this study. The solvents water and alcohol commonly used in the field were replaced in this study by distilled water and 70% ethanol. Extraction of secondary metabolites from these solvents showed that the ethanol-water mixture (70/30, v/v) gave the best yield. The anti-candidus study of these different plant extracts highlighted the antifungal parameters of the plants on a multi-resistant strain of *candida albicans*. The study showed that *T. ivorensis* was the plant that gave the best results on the strain tested. Nonetheless, the present study initiates further research on the hydroethanol extract of *Terminalia ivorensis* with the objective of fractionating and determining the structure of the molecule of antifungal interest.

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