Evaluation of the Antifungal Activity of Five Medicinal Plants on the *in vitro* Growth of a Multi-resistant Strain of *Candida Albicans*

Konan Gbê Kouakou N'dri Ange
Ouattara Sitapha
Biology and Health Laboratory, UFR Biosciences,
Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire
Kanga Yao
Ethnobotanical-Ethnopharmacological Laboratory, UFR Sciences Biologiques, Université Peleforo Gon Coulibaly, Korhogo, Côte d'Ivoire
Monney Yapo Urbain
Kra Adou Koffi Mathieu
Biology and Health Laboratory, UFR Biosciences,
Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire

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Abstract

This study was conducted to determine the antifungal potential of plants traditionally used in the treatment of 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 the 70% ethanolic extracts were more active than the aqueous extracts for all plants. The 70% ethanolic extract of *T. ivorensis* (FMC = 1.56 mg/ml) was the most active on the tested strain. It was followed by *T. superba* (MFC = 3.125 mg/ml) *D. benthamianus* (MFC = 50 mg/mL), *G. arborea* (MFC= 50 mg/mL) and *J. secunda* (MFC > 50 mg/mL). The phytochemical screening revealed the presence of seven major groups of compounds, among which saponins and catechins are the

most abundant in the aqueous extracts. Polyphenols, Flavonoids, Polyterpenes and Sterols as well as Tannins are equally present in the alcoholic and aqueous extracts of the different plants at variable levels. These compounds could justify its activity and its traditional use.

Keywords: Phytochemical screening, Antifungal activity, Medicinal plants

Introduction

Fungal infections have increased dramatically in recent years and have become a major public health concern (Gudlaugsson *et al.,* 2003). The *Candida* genus includes commensal microorganisms, which are the cause of the majority of opportunistic infections (Develoux and Bretagne 2005; Kettani *et al*., 2006). Their pathogenicity is particularly evident in the presence of favourable 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 caused by fungal infections (Lin *et al*., 2001), hence the interest in searching for new antifungal agents of natural origin. Just as certain micro-organisms such as the genera *Penicillium* or *Streptomyces have been* used as sources of antimicrobials, certain plants have been used for centuries in traditional medicine to fight infections. Even today, in some Asian and African countries, 80% of the population uses medicinal plants to treat various health problems (Ackah, 2004). Therefore, our study focused on these plants to contribute to the search for new antimicrobials. Among the numerous plant species identified in Côte d'Ivoire, five plants were selected for study (Kra *et al.,* 2014). Aqueous decoctions or macerations (aqueous and alcoholic) of the different organs of these plants are used in Côte d'Ivoire and other countries in sub-Saharan Africa to combat diarrhoea, skin infections (Adjanohoun and Aké Assi 1979). The aim of this study is to determine the chemical composition of the two extracts from each plant and to evaluate their antifungal activity on a multi-resistant strain of *candida albicans.*

Materials and methods

Plant material

The various plant organs were harvested in April 2021 in southern, eastern and central Côte d'Ivoire. They were then sorted, washed, cut into small pieces and dried in the sunlight for 21 days before being ground into a fine powder.

Table 1. Organ taken from each plant

Plants	Organ removed	Harvest area
Distémonanthus benthamianus	Trunk bark	Abengourou
Gmelina arborea	Trunk bark	Abidjan
Justicia seconda	Whole plant	Abidjan
T. ivorensis	Trunk bark	Agboville
T. 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	FCB^R , IT^R , VRC^R	Sabouraud without chloramphenicol	30°C	48

Plant extraction

The different parts of the plants were harvested, cut up and dried in an air-conditioned room at 18°C for one month. 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 litre of distilled water in a blender. After six grinding cycles, the homogenate obtained in each case was first wrung out in a clean 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 colour from one plant to another obtained constitutes the total aqueous extract. The hydroalcoholic extract was prepared by the same procedure 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

We characterised the different chemical groups with reference to the techniques described in the work of Békro *et al*.

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

The reaction with ferric chloride (FeCl3) was used to characterise the polyphenols. To 2 ml of each extract (aqueous and 70% ethanol), we added a drop of 2% alcoholic ferric chloride solution. 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. On addition of 2-3 magnesium chips, there is a release of heat and then a pinkish-orange or purplish coloration. The addition of 3 drops of isoamyl alcohol intensified this coloration which confirmed the presence of flavonoids.

Catechic tannins 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 min. The observation of a coarse flake precipitate characterised the catechic tannins. For the gallic tannins, we filtered the previous solution. The filtrate was collected and saturated with sodium acetate. The addition of 3 drops of FeCl3 would cause the appearance of an intense blue-black coloration, a sign of the presence of gallic tannins.

The quinone substances were determined using Bornstraëgen's reagent. Two (2) ml of each of the 3 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. The triturate was then heated in a water bath for 30 minutes. After cooling, it is extracted with 20 ml of chloroform. Ammonia diluted 2 times (0.5 mL) was added to the chloroform solution. A red or purple colouration 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. 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 the total aqueous extract was poured into a test tube. The tube was shaken for 15 s and then left to stand for 15 min. A persistent foam height of more than 1 cm indicated the presence of saponosides.

Antifungal test

From a young *Candida albicans* culture (48 hours), the inoculum was prepared as follows: A young colony of *Candida albicans* collected with a loop was homogenised in 10 mL of sterilised distilled water. This yielded the

stock suspension (10^0) concentrated to 10^6 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 10^5 cells/ml. For each of the test tubes of each series of eighteen extracts (except for the sterility control tube of the culture medium), the culture of the germs was carried out on the previously prepared media by seeding 10 µl of the 10^{-1} suspension in transverse streaks until exhaustion. This corresponds to 1000 seeded cells. The cultures thus produced were incubated at 30°C for 48 hours. After 48 hours of incubation, *Candida albicans* colonies were counted by direct counting with a colony counting pen. Growth in the eight experimental tubes of each series was assessed as percentage survival calculated against 100% survival in the growth control tube (Ouattara *et al.*, 2013a).

Result

Extraction efficiency

Benthamianus, 15.29 ±1.33 and 9.67 ±1 for *G. arborea*, 27.9 ±1 and 19.15 ±1 for *J. secunda*, 28.29 ±1.14 and 19.48 ±1 for *T. ivorensis*, 32.58 ±0.12 and 22.56 ±2.1 for *T. superba*. The aqueous extracts of the different plants recorded the best yields compared to the hydroethanol extract. *T. superba* was the highest yielding plant with both the aqueous and the 70% ethanolic extracts. *G. arborea* recorded the lowest yield.

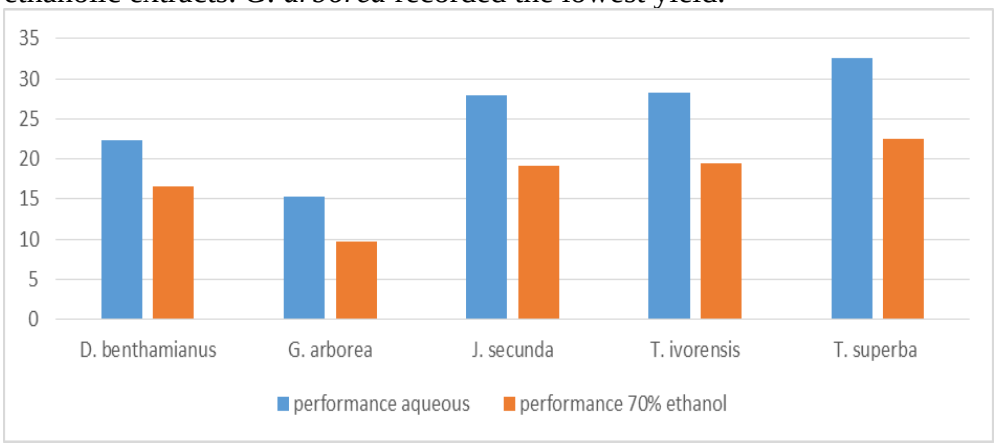

Figure 1. Extraction performance

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 gall tannins and quinones was observed in the *J. secunda* extracts. The extracts of D 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 poly terpenes	Polyphenol compounds	Flavonoids	Tannins		Quinones	Alkaloids		Saponosides
					Gallics	Catechism		burchard	dragendorff	
D. benthamianus	Aqueous	+	+	+	+	-	-	+	-	+
	70% ethanol	++	+	+	+	-	-	+	-	-
J. secunda	Aqueous	+	+	+	-	-	-	+	+	+
	70% ethanol	+	+	+	-	+	-	+	+	-
G. arborea	aqueous	+	+	+	+	+	+	+	+	+
	70% ethanol	+	+	+	+	+	+	+	+	-
T. ivorensis	aqueous	+	++	++	+	+	+	+	+	++
	70% ethanol	+	++	++	+	+	+	+	+	-
T. superba	aqueous	+	+	++	+	+	+	+	+	++
	70% ethanol	+	+	++	+	+	+	+	+	-

-: Absent, +: Present; ++: Abundant;

Antifungal activity

The anti-candidus activity carried out allowed the determination of the IC_{50} and the MFC of each plant extract. The antifungal parameters of the different plants are shown in Table II. 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 D. benthamianus (IC_{50} =5.2 mg/mL; FMC>50 mg/mL) and the most active extract is the hydroethanolic extract of *T. ivorensis* (IC_{50} =0.24 mg/mL; FMC=1.56 mg/mL)

Table 4. Antifungal parameters of plant extracts

Plants		*Candida albicans*	
		Antifungal parameters in mg/mL	
		CI_{50}	CMF
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

We studied the antifungal properties of five plants used in traditional medicine. 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. Sterols, polyterpenes, flavonoids and phenolic compounds are present in all nine plant extracts with a relative abundance in the extracts of *terminalia ivorensis and terminalia superba*. The seven metabolites were found in *G. arborea* extracts, *terminalia, ivorensis, terminalia, superba* extracts at variable levels. 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 (Table). These results reveal that *A. occidentale* has no activity on the multi-resistant strain of *candida albicans* tested. However, a reduction in the percentage of survival of *candida albicans was* observed at high concentrations.

From the comparison of the antifungal parameters (**Table 4**) 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, a conclusion consolidated by the work of Kra *et al*.

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

When we compare the performance of the hydroethanolic extract of *T. ivorensis* with hydroalcoholic extracts from other works, it appears that the extracts of *T. ivorensis* have the best antifungal activities. Indeed, the analysis reveals that the hydroethanolic extract of *T. ivorensis* (MFE=1.56mg/mL) is 16 times more active than the 70% ethanolic extract of *Morinda morindoides* (MFE=25mg/mL) [13], 256 times more active than the extract of *Entandrophramgma cylindrium* (MFE=800mg/mL), 64 times more active than the hydroalcoholic extract of *Khaya ivorensis* (MFE=100mg/mL) (Kra et al., 2014)

The different antifungal parameters can be improved by fractionating or partitioning the most active extracts.

Conclusion

Five plants used in traditional settings for microbial diseases were used 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) is the best extractant. The anti-candidus study of these different plant extracts allowed to highlight 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. The present work initiates further work 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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