

## Anticandidosis activity of aqueous and ethanolic extracts of *Harungana madagascariensis*, *Zanthoxylum leprieurii* and *Xylopiya aethiopia*

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### Abstract

### Original Research Article

Some ailments have been cured either by the use of plant parts or by mixing different plant parts (recipes). It is with this in mind that this study originated, focusing on the valorization of plants from the African pharmacopoeia. Thus, the activity of single extracts and aqueous and hydroalcoholic mixtures of the bark and leaves of *Harungana madagascariensis*, the bark of *Zanthoxylum leprieurii* and the fruit of *Xylopiya aethiopia* was evaluated on the *in vitro* growth of *Candida* strains (*Candida albicans* 18650 and *Candida albicans* 18792 and *Candida glabrata*). Antifungal tests were carried out by plating 1,000 cells of each fungal germ on Sabouraud agar medium using the double dilution slant tube method. Deth70% extract inhibited the growth of *Candida albicans* 18650 and *Candida albicans* 18792 at an FMC value of 25 mg/mL, and *Candida glabrata* at an FMC value of 50 mg/mL. Phytochemical screening revealed the presence of chemical compounds such as polyphenols, flavonoids, saponosides, gallic and catechic tannins and the absence of sterols.

**Keywords:** Antifungal tests, *Harungana madagascariensis*, *Zanthoxylum leprieurii*, *Xylopiya aethiopia*.

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## INTRODUCTION

In developing countries, infectious diseases are a major public health concern due to their frequency and severity (Bourgeois, 1999). Indeed, they are responsible for over 17 million deaths per year worldwide, more than half of which occur on the African continent alone (WHO, 2006). In Côte d'Ivoire, studies carried out between 1996 and 2000 revealed that 52.12% of all dermatological consultations were caused by yeasts (Attoh-Touré *et al.*, 2009). What's more, human fungal pathogens are becoming as important because of mortality as hospital-acquired infections (Sternberg, 1994). At the same time, the treatment of mycoses is becoming increasingly difficult due to the development of resistance to the usual antibiotics. Indeed, many molecules derived from chemical therapeutics are increasingly ineffective in the treatment of certain mycoses, including candidiasis. These resistance phenomena are due to mutations, long-term antibiotic therapy and environmental pressures, etc (Dromer & Dupont, 1996).

Faced with this situation, the search for new therapeutic alternatives is imperative. One way of thinking about this is the use of medicinal plants. In Africa, over 80% of the population rely exclusively on plants as sources of medicinal treatment, especially in the fight against infectious diseases. The use of these plants is justified by cultural habits, people's purchasing power and the accessibility of plant resources. In the case of mycoses, several plant species are used in traditional medicine, including *Cassia alata*, *Mitracarpus scaber*, *Eucalyptus torrelianna*, *Termiliana superba*, *Harungana madagascariensis*, *Zanthoxylum leprieurii* and *Xylopiya aethiopia*. In the Bongouanou region of Ivory Coast, *Harungana madagascariensis*, *Zanthoxylum leprieurii* et *Xylopiya aethiopia* *Harungana madagascariensis*, *Zanthoxylum leprieurii* and *Xylopiya aethiopia* are used in the preparation of various recipes for the treatment of skin diseases. This ethnomedicinal use of these plants suggests that a scientific study should be carried out on them, in order to determine their true antifungal potential. It is within this framework that the present

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study has been carried out. Its overall aim is to contribute to the valorization of traditional Ivorian pharmacopoeia plants by collecting data on their efficacy.

## 2 Plant material

The plant material consisted of the leaves, fruit and bark of three plants (*Harungana madagascariensis*, *Zanthoxylum leprieurii*, et *Xylophia aethiopica*).

### Fungal strains

The fungal material consisted of clinical strains of: *Candida albicans* 18650, *Candida albicans* 18792 and *Candida glabrata*. These germs were supplied by the mycology laboratory of the Institut Pasteur of Ivory Coast.

### Culture medium

Antibiotic-free Sabouraud agar was used as a culture medium for the fungal germs studied. This medium was supplied by Biomedis.

### Plant collection

Bark from the trunk of *Harungana madagascariensis* and *Zanthoxylum leprieurii*, the fruits of *Xylophia aethiopica* and the leaves of *Harungana madagascariensis* were harvested in March 2022 in the Bongouanou region (Ivory Coast). Samples of this harvest were packed in bags. They were identified at the National Floristic Center (NFC) of the Université Félix Houphouët-Boigny (UFHB). The bark, leaves and fruit were cut into small pieces and dried in the shade at an ambient temperature of  $27\pm 2^\circ\text{C}$  at the Biochemistry Laboratory of the Université Jean Lorougnon Guédé (UJLG) in Daloa for around three weeks. Once dried, they were reduced to fine powders using an electric grinder. The powders were used to prepare the various aqueous and ethanolic extracts.

### Preparation of plant extracts

Plant extracts were prepared according to the method of (Zirih *et al.*, 2003). For this purpose, 100 g of plant powder were homogenized in 1L of distilled water using an electric mixer. The resulting homogenate was wrung out in a clean cloth square, followed by successive triple filtration on absorbent cotton. The aqueous filtrate thus obtained was concentrated to 2/3 using a BUCHI-type rotary evaporator at a temperature of  $60^\circ\text{C}$ . The concentrated solution corresponding to 1/3 of the initial solution was frozen and freeze-dried to give a powder which constitutes the aqueous extract. The ethanolic extract was prepared using the same procedure as described above, with the difference that the solvent used was a mixture of ethanol (70%) and distilled water (30%) v/v. The filtrate was concentrated to dryness using a BUCHI-type rotary evaporator at  $60^\circ\text{C}$  to give a powder which constitutes the ethanolic extract.

### Extract composition

Extract M1 (aqueous and ethanolic) is derived from a mixture of 40% *Harungana madagascariensis*

leaf powder, 50% *Zanthoxylum leprieurii* powder + bark and 10% *Xylophia aethiopica* fruit powder. Extract M2 (aqueous and ethanolic) is extracted from a grind consisting of 40% *Harungana madagascariensis* bark powder, 50% *Zanthoxylum leprieurii* powder + bark and 10% *Xylophia aethiopica* fruit powder. Extract D (aqueous and ethanolic) is derived from 100% *Harungana madagascariensis* bark powder, while extract A (aqueous and ethanolic) is derived from 100% *Harungana madagascariensis* leaf powder. The difference between M1 and M2 is that the former contains the leaves of *Harungana madagascariensis*, while the latter contains the bark from the trunk of the same plant. Extract B (aqueous and ethanolic) is made up of 100% *Zanthoxylum leprieurii* bark powder and extract C (aqueous and ethanolic) is made up of 100% *Xylophia aethiopica* fruit powder.

### Calculating yields

Yield is the quantity of extract obtained from a plant material (Bssaibis *et al.*, 2009). It is expressed as a percentage of dry matter (plant powder) and is calculated using the formula:

$$R(\%) = \frac{M1}{M0} \times 100$$

R(%): Extract yield expressed as a percentage (%);

M1: Mass of extract (in g);

M0: Mass of vegetable powder (in g).

### Antifungal tests

#### Preparation of Sabouraud agar

Antibiotic-free Sabouraud agar was prepared by dissolving 4.96 g in 80 mL distilled water. The resulting mixture was heated and stirred on a magnetic stirrer until the agar was completely dissolved. The prepared culture medium was dispensed into test tubes.

#### Incorporating plant extracts into agar

Plant extracts were incorporated into the agar using the double dilution method in inclined tubes (Guédé-Guina *et al.*, 1997). Each series comprises seven test tubes numbered 1 to 7. The previously prepared medium was distributed among the seven tubes, with 20 mL in tube no. 1 and 10 mL in the other tubes, from no. 2 to no. 7. The concentration range was prepared by homogenizing 4 g of aqueous extract powder or ethanolic extract in 20 mL in tube no. 1. Half the volume of this homogeneous mixture was transferred to tube no. 2 containing 10 mL agar and homogenized. This operation was repeated for tube no. 3 and so on by double dilution up to tube no. 5, where half the volume of the homogeneous mixture was discarded. This gave a concentration range from 200 mg/mL to 12.5 mg/mL with a geometric bond of reason  $\frac{1}{2}$ . Tube no. 6 was used for germ growth control in the absence of plant extract (TC), and tube no. 7 for agar sterility control in the absence of germ (TS) and extract. The seven tubes in this series were sterilized in an autoclave at  $121^\circ\text{C}$  for 15 min

(Adeshina *et al.*, 2012), then inclined (with a small pellet) at room temperature to allow solidification on the agar.

### Inoculum preparation

For antifungal tests, inocula were prepared separately from 48-hour-old cultures of the three *Candida* species on slant agar. For each species, a well-isolated colony was picked with a 2 mm loop and homogenized in 10 mL of sterilized distilled water. This suspension yielded a stock suspension rated  $10^8$ , with a load of 106 cells/mL. From suspension  $10^8$ , suspension  $10^{-1}$  was prepared by 1/10th dilution, transferring 1 mL of suspension  $10^8$  into 9 mL of sterilized distilled water to give a final volume of 10 mL, containing 105 cells per mL (Zirih *et al.*, 2003).

### Inoculation and incubation

For each series, with the exception of tube no. 7 for sterility control, the various fungal species (*Candida albicans* 18650, *Candida albicans* 18792 and *Candida glabrata*) were cultured in the other tubes. Inoculation was carried out in transverse streaks (until exhaustion) of 10  $\mu$ L of suspension  $10^{-1}$  (concentration  $10^5$  cells/mL), giving 1000 inoculated cells. The resulting cultures were incubated at 37°C for 48 hours for all *Candida* species (Zirih *et al.*, 2003; Dewanjee *et al.*, 2007).

### Determination of antifungal parameters

MICs were determined by comparing the percentages of strain growth in the experimental tubes to

the control tubes. The concentration of the experimental tube with no visible growth (no visible colonies) corresponds to the MIC. The MFC is determined following the MIC. A subculture of the tube identified as carrying the minimum inhibitory concentration is performed on fresh agar (no plant extracts). The agar surface is scraped with the loop and inoculated onto this agar. After 24 hours' incubation, the FMC is read in the tube showing no growth visible to the naked eye (Guédé-Guina *et al.*, 1996).

## RESULTS

### Yields of the various extracts obtained

The average masses obtained with an initial mass of 100 g of fine powder are given in Table I. Analysis of this table shows that the highest yields were obtained with aqueous extracts D (12.1%) and C (10.65%). Extract B, on the other hand, gave the lowest extraction yield (3.62%). Extracts from the mixture (M1 and M2) and extract A had extraction yields (M1: 6.85%; M2: 5.98% and A: 7.82%) between high values (D: 12.1% and C: 10.65%) and low values (B: 3.62%). For ethanolic extracts with an initial mass of 100 g, the average masses obtained were relatively high. Extracts D (21.66%) and C (13.68%) obtained high yield values. The lowest yield was again obtained with extract B (1.88%). The other extracts A (11.38%), M1 (6.46%) and M2 (6.68%) had yield values ranging from the highest (D: 21.66%) to the lowest (B: 1.88%).

**Table I: Yields of aqueous and hydroethanolic extracts of the three plants**

Average mass (g)	Aqueous extract						Hydroethanolic extract					
	A	B	C	D	M1	M2	A	B	C	D	M1	M2
	7,82	3,62	10,65	12,10	6,85	5,98	11,38	1,88	13,68	21,66	6,46	6,68
Yield	7,82	3,62	10,65	12,10	6,85	5,98	11,38	1,88	13,68	21,66	6,46	6,68

Aaq: *H.madagascariensis* leaf, Baq: *Z. leprieurii* bark; Caq: *X. aethiopica* fruit; Daq: *H.madagascariensis* aqueous bark; M1aq: blend 1; M2aq: blend 2

Aeth 70%: Leaf of *H.madagascariensis*, Beth 70%: Bark of *Z. leprieurii*, C eth 70%: Fruit of *X. aethiopica* Deth 70%: Bark of *H.madagascariensis* ethanolic 70% ; M1eth 70%: blend 1, M2eth 70%: blend 2

### Tri-phytochemical study

The results of the phytochemical screening tests are shown in Table II below. These tests revealed certain chemical compounds, namely polyphenols, flavonoids, saponosides, gallic tannins and catechic tannins in all extracts (aqueous and ethanolic) of three plants. The

presence of anthocyanins was revealed in extracts Aaq; Baq; Caq; Daq; Aeth70%; Beth70%; Ceth70 and M1eth70%; but absent in extracts M1aq; M2aq; Deth70% and M2eth70%. None of the extracts contained sterols (Table II).

**Table II: Tri-phytochemical analysis of aqueous and hydroalcoholic extracts**

Chemical group							
Plants	Polyphenols	Flavonoids	Saponosides	Gallic tannins	Catechic tannins	Sterols	Anthocyanins
Aaq	+	+	+	+	+	-	+
Baq	+	+	+	+	+	-	+
Caq	+	+	+	+	+	-	+
Daq	+	+	+	+	+	-	+
M1aq	+	+	+	+	+	-	-
M2aq	+	+	+	+	+	-	-
Aeth70%	+	+	+	+	+	-	+

Chemical group							
Plants	Polyphenols	Flavonoids	Saponosides	Gallic tannins	Catechic tannins	Sterols	Anthocyanins
Beth70%	+	+	+	+	+	-	+
Ceth70%	+	+	+	+	+	-	+
Deth70%	+	+	+	+	+	-	-
M1eth70%	+	+	+	+	+	-	+
M2eth70%	+	+	+	+	+	-	-

Presence: (+) Absence: (-) ;

Aaq: Leaf of *H.madagascariensis* Baq: Bark of *Z. lepreurii*; Caq: Fruit of *X. aethiopica*; Daq: Bark of *H.madagascariensis*; M1aq: mixture 1; M2aq: mixture 2; Aeth 70%: Leaf of *H. madagascariensis*; Beth 70%: *Z. lepreurii* bark; Ceth 70%: *X. aethiopica* fruit; Deth 70%: *H.madagascariensis* bark; M1eth 70%: blend 1; M2eth 70%: blend 2

### Effects of extracts on in vitro growth of germs studied

#### Action of aqueous extracts on fungal germs.

Experimental data on the antifungal activity of aqueous extracts on the in vitro growth of fungal germs (*C. albicans* 18650; *C. albicans* 18792 and *C. glabrata*) showed a decrease in the number of colonies of fungal species as the concentration of aqueous extracts increased in experimental tubes compared with the growth control. The antifungal parameters of the aqueous extracts are shown in Table III. Analysis of the antifungal parameters obtained with the aqueous extracts shows that they exerted variable antifungal activities on the in vitro growth of the fungal germs studied. Of all the aqueous extracts tested, the Daq extract inhibited in vitro growth

at 50 mg/mL of *C. albicans* 18650 and *C. albicans* 18792 and at 100 mg/mL of *C. glabrata*. Extracts (Baq and M2aq) were moderately active on the growth of *C. albicans* 18650, while extracts (Caq and M1aq) were weakly active at CMF=100 mg/mL on the same germ. The action of extracts (Aeth and M1eth) on the growth of *C. albicans* 18792 germs shows that these extracts had a more significant activity (FMC=50 mg/mL) than extracts (Baq; M2aq; Caq) with respective FMCs (100mg/mL; 100mg/mL; 200mg/mL). Thus, *C. glabrata* would be the germ whose action of the extracts (Aaq; Baq; Caq; M1aq and M2aq) is weakly appreciated with a MFC=200 mg/mL.

**Table III: Antifungal parameters of aqueous plant extracts**

Extracts	<i>C. albicans</i> 18650	<i>C. albicans</i> 18792	<i>C. glabrata</i>
	FMC (mg/mL)	FMC (mg/mL)	FMC (mg/mL)
Aaq	100	50	200
Baq	50	100	200
Caq	100	200	200
Daq	50	50	100
M1aq	100	50	200
M2aq	50	100	200

Aqueous extracts Aaq: Leaf of *H.madagascariensis*; Baq: Bark of *Z. lepreurii*; Caq: Fruit of *X. aethiopica* ; Daq: *H.madagascariensis* bark; M1aq: blend 1; M2aq: blend 2

#### Action of hydroalcoholic extracts on fungal germs

Compared with the growth control tube, the number of colonies decreased as plant extract concentrations increased in the experimental tubes.

All the ethanolic extracts tested exerted an inhibitory action on the *in vitro* growth of the three candida germs. However, on the basis of the FMC values obtained, the Deth70% extract obtained the lowest FMC values. These values are 25 mg/mL on *Candida albicans* (18650 and 18792) and 50 mg/mL on *C. glabrata*. In contrast, the extracts with the highest FMC values were

Aeth70%; Beth70% and Ceth70%. FMC values for these extracts are 100 mg/mL on *C. albicans* (18650 and 18792) and 200 mg/mL on *C. glabrata*. Extracts M1 eth70% and M2 eth70%) from mixtures obtained CMF FMC values of 50 mg/mL and 100 mg/mL respectively on the growth of *Candida albicans* (18650 and 18792) and *C. glabrata*.

*C. glabrata* was the most difficult fungus to inhibit. High FMC values (200 mg/mL, 100 mg/mL and 50 mg/mL) were obtained on *in vitro* growth of this fungal germ (Table IV).

**Table IV: Antifungal parameters of ethanolic plant extracts**

Extracts	<i>C. albicans</i> 18650	<i>C. albicans</i> 18792	<i>C. glabrata</i>
	FMC (mg/mL)	FMC (mg/mL)	FMC (mg/mL)
Aeth70%	100	100	200
Beth70%	100	100	200
Ceth70%	100	200	200

Extracts	<i>C. albicans</i> 18650	<i>C. albicans</i> 18792	<i>C. glabrata</i>
	FMC (mg/mL)	FMC (mg/mL)	FMC (mg/mL)
Deth70%	25	25	50
M1eth70	50	50	100
M2eth70	50	50	100

Ethanol extracts: Aeth70%: *H.madagascariensis* leaf, Beth70%: *Z. leprieurii* bark, Ceth70%: *X. aethiopica* fruit, Deth70%: *H.madagascariensis* bark, M1eth70%: blend 1; M2eth70%: blend 2

### Comparison of the action of aqueous and ethanolic extracts on fungal growth

The FMC antifungal parameter values obtained in this study enable a comparison to be made between the extracts (aqueous and ethanolic) tested and the fungal germs *Candida albicans* (19650 and 18792) and *C. glabrata*. This comparison showed that extract D eth had the best activity on all the germs tested. This extract obtained the lowest FMC values (50 mg/mL and 25 mg/mL) on the growth of *Candida albicans* (19650 and

18792) and *C. glabrata* respectively. The less active extract was Caq. It recorded the highest FMC values (FMC =100 mg/mL on the growth of *C. albicans* 19650 and FMC =200 mg/mL on the growth of *Candida albicans* 18792 and *C. glabrata*). The other extracts exerted inhibitory actions intermediate between the Detn and Caq extracts. Among fungal germs, *Candida glabrata* was the most resistant to the inhibitory actions of all extracts. The FMC values obtained on this germ are high (50 mg/mL to 200 mg/mL) Table V.

**Table V: Antifungal parameters of aqueous and ethanolic plant extracts**

Extracts	Fungal germs					
	<i>Candida albicans</i> 19650		<i>Candida albicans</i> 18792		<i>Candida glabrata</i>	
	FMC (mg/mL)		FMC (mg/mL)		FMC (mg/mL)	
	aqueous	ethanolic	Aqueous	Ethanolic	Aqueous	ethanolic
A	100	100	50	100	200	200
B	50	100	100	100	200	200
C	100	100	200	200	200	200
D	50	25	50	25	100	50
M1	100	50	50	50	200	100
M2	50	50	100	50	200	100

Aqueous extracts Aaq: Leaf of *H.madagascariensis*; Baq: Bark of *Z. leprieurii*; Caq: Fruit of *X. aethiopica* ; Daq: *H.madagascariensis* bark; M1aq: blend 1; M2aq: blend 2

Ethanol extracts: Aeth70%: *H.madagascariensis* leaf; Beth70%: *Z. leprieurii* bark; Ceth70%: *X. aethiopica* fruit; Deth70%: *H.madagascariensis* bark; M1eth70%: blend 1; M2eth70%: blend 2

## DISCUSSION

The search for a new molecules to combat infectious agents remains a major concern for today's researchers. Indeed, microorganisms are developing resistance to synthetic drugs. Faced with this problem, the search for new processes for the acquisition of new therapeutic agents, without side effects or undesirable effects, would be desirable while enhancing the *Harungana madagascariensis*, *Zanthoxylum leprieurii* et *Xylopi aethiopica* value of pharmacopoeial plants. It is against this backdrop that this study takes shape, focusing on with antimicrobial properties with two types of mixtures in different proportions and extracts (aqueous and ethanolic) of each plant. The anti-infectious properties were attributed both to the individual plants and to the blends.

The extraction yields of the various solvents with the three plants studied (leaf and bark of *Harungana madagascariensis* ; bark of *Zanthoxylum leprieurii* and the fruits of *Xylopi aethiopica*) showed significant differences. Among the extracts, ethanolic extract D derived from *Harungana madagascariensis* bark powder showed the highest yield (21.66%), while extract B

derived from *Zanthoxylum leprieurii* bark powder recorded the lowest yield (1.88%). Similarly, aqueous extracts D and B always showed high yields (12.1%) for extract D and low yields for extract B. In general, the yields of ethanolic extracts are higher than those of aqueous extracts. These results are similar to those of several other authors who also showed that extracts from the ethanol-water mixture (70/30; v/v) gave high yields than extracts from water alone (Ahon *et al.*, 2012; Ahon *et al.*, 2022).

Analysis of extraction capacity shows that the water/ethanol solvent mixture extracts active ingredients better than the water-only solvent. The variability observed in extraction yields is probably linked to the chemical composition of the plants used. In addition, this difference in extraction capacity could depend on the affinity of the solvent for the phytochemicals, on the one hand, and on the polarity of the solvent, on the other (Dah-Nouvlessounon *et al.*, 2015).

After extraction, each extract was evaluated for in vitro growth of fungal germs. The antifungal test showed that the Minimum Fungicidal Concentration

(MFC) values of the different extracts tested varied from one germ to another and from one extract to another. Aqueous extracts (Aaq; Baq; Caq; Daq; M1aq and M2aq) showed varying activity. Indeed, of all the aqueous extracts tested, only the Daq extract showed the best inhibition with a Minimum Fungicidal Concentration (MFC) of 50 mg/mL on the growth of *Candida albicans* 18650 and *Candida albicans* 18792 and 100 mg/mL on *Candida glabrata*. These results differ from those of Onzo *et al.*, (2015).

In their work, these authors showed that aqueous extracts of four plants (*Thalia geniculata*, *Musa spp*, *Manihot esculenta* and *Daniellia oliver*) had no activity on the *in vitro* growth of *Candida albicans* at a dose of 100 mg/mL. This difference in results could be due to the harvesting period or area, the extraction method, the concentration of active ingredient and the nature of the fungal germs used Thangara *et al.*, (2000). Unlike Daq extract, Baq extract exerted a weak inhibition on the growth of all fungal germs. These results can be explained by the level of sensitivity of each fungal germ. In fact, some germs are less sensitive, others moderately sensitive and others highly resistant. Like the aqueous extract, ethanolic extracts showed satisfactory results in this study. Six ethanolic extracts were tested for antifungal activity. The different extracts showed varying degrees of activity against the *in vitro* growth of the germs studied. However, the Deth extract showed stronger inhibitory activity on germ growth than the others. This inhibitory activity was 50 mg/mL on *Candida albicans* 18650 and *Candida albicans* 18792, and MFC =25 mg/mL on *Candida glabrata*. The high antifungal activity recorded with the latter demonstrates that the secondary metabolites contained in this extract (Deth70) have greater antifungal potential. These results are similar to those of Ouattara *et al.*, (2007) who also showed that ethanolic extracts of *Morinda morindoides* and *Thonningia sanguinea* exhibit better antifungal activity than the aqueous extract. In contrast to Deth70 extract, germs were less sensitive to the inhibitory action of B eth extract. The highest MFC values were obtained with it (100 mg/mL on *Candida albicans* 18650 and 200 mg/mL on *Candida albicans* 18792 and *Candida glabrata*).

These results can be explained by the fact that the hydroalcoholic solvent (ethanol/water) has the potential to extract metabolites better than the aqueous extract. The work of Kra *et al.*, (2014); Ahon *et al.*, (2022) is similar to ours. These authors have shown that the hydroalcoholic solvent (ethanol/water) better concentrates the small-molecular-weight substances with better antifungal activity, which would otherwise be masked by the large-molecular-weight chemical molecules in the aqueous extract. The hydroethanol extract has a more pronounced action on *C. albicans* than the aqueous extract. These results were observed by Paris & Moyses, 1965. Indeed, these authors demonstrated that *C. albicans* is sensitive to the action of *M. charantia*,

which was also observed in this study. Fungal germs (*Candida glabrata*, *Candida albicans* 18650 and *Candida albicans* 18792) were all sensitive to the various extracts. What's more, several authors have revealed that mixed solvents are highly effective at extracting compounds (Paris & Moyses, 1965). They even went so far as to say that the use of mixed solvents results in a high enrichment of extracts in compounds. The superiority of mixed solvents is said to be due to the increased solubility of chemical compounds in extracts obtained with mixed solvents compared to those obtained with pure solvents (Handa *et al.*, 2008).

The hydroalcoholic extracts (M1eth70 and M2eth70) both showed the same germ-specific results. Growth inhibition of *albicans* 18650 and *C. albicans* 18792 at 50 mg/mL and 100 mg/mL MFC was observed on *C. glabrata*. This might suggest that the mixtures contain the same types of chemical compounds. This hypothesis was supported by the same inhibitory activity on the *in vitro* growth of *albicans* 18650; *C. albicans* 18792 as *C. glabrata*. The difference in inhibition between the extract of the mixtures and the simple hydroalcoholic Deth70 extract could be explained by the fact that, during the extraction process, the antimicrobial activity was potentiated by certain molecules present in the simple ethanolic extract and diminished either by the formation of other antagonistic molecules or by the disappearance of a considerable number of these molecules with antifungal activity in the mixtures. Observing the activity of the leaf and bark of *H. madagascariensis*, inhibition was highest in the bark of this plant. This suggests that *H. madagascariensis* bark contains more active compounds than those found in the leaves.

Taking into account all aqueous and ethanolic extracts, the activity of extracts from the mixed water/ethanol solvent proved more effective than their equivalents from the water solvent. On the same plant, the activity of the extracts varied according to the part of the plant extracted. Aqueous and ethanolic extracts of *H. madagascariensis* bark showed a high level of activity, irrespective of the solvent used. These results may be explained by the presence of more compounds responsible for growth inhibition in the bark than in the leaves.

Given that the extract (Deth70%) showed the best inhibition against all the germs tested, this shows that the mixed solvent water/ethanol is the best solvent par excellence in this study. This finding is shared by the work of Traoré *et al.*, (2012), on the antifungal and antibacterial activity of *Annona senegalensis* leaves suggested that ethanol was a better solvent than water (Traoré *et al.*, 2012). To continue, subculture of these germs revealed their growth after 48 hours of incubation. The activity of ethanolic extract of *H. madagascariensis* bark is fungistatic.

All the inhibitory activities of the extracts on the growth of fungal germs were exerted by the chemical molecules contained in these extracts. Their presence was revealed by phytochemical studies. This study showed that the aqueous and hydroalcoholic extracts contained major chemical groups of molecules such as saponosides, flavonoids, polyphenols and tannins. The absence of certain major chemical groups such as sterols may be due to certain anthropogenic factors such as a difference in several parameters either geographical, physicochemical or biological, such as the difference in the harvesting site including the plant environment, light, precipitation, topography, season, soil type, harvesting period, genetic heritage, extraction procedure used, part of the plant studied or their phytochemicals (Jayasinghe *et al.*, 2003). The presence of compounds such as saponosides, flavonoids, polyphenols and tannins could be at the root of the extracts' antimicrobial power. The antimicrobial activity of these compounds has already been confirmed by several authors (Jayasinghe *et al.*, 2003; Konate *et al.*, 2012). These secondary metabolisms are capable of disrupting microbial membranes, leading to cell death. Furthermore, Lambert *et al.*, (2001) have shown that phenolic compounds cause an increase in plasma membrane permeability, leading to a change in pH and the release of organic ions.

## CONCLUSION

The present study assessed the antifungal activity of aqueous and ethanolic extracts of *H. madagascariensis*, *Z. leprieurii* and *X. aethiopica*. The results showed that, whatever the type of solvent used, the single extracts were more active than the mixtures. The most active extracts were the aqueous Daq extract and the ethanolic extract. These extracts inhibited the *in vitro* growth of all fungal germs. However, the activity of the Deth70% extract was stronger on all fungal germs. To further enrich this study, the molecule(s) responsible for the antifungal activity of Deth70% ethanolic extract should be identified.

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