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Original Research

Anti-fungal activities of medicinal plants extracts of Ivorian pharmacopoeia

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ABSTRACT

Aim: This study was to evaluate *in vitro* anti-fungal activity of aqueous and hydroethanolic from medicinal plants extracts collected in Côte d'Ivoire. **Materials and Methods:** Plants extracts were prepared by homogenization and separately incorporated to Sabouraud agar using the agar slanted double dilution method. Ketoconazole was used as standards for anti-fungal assay. The anti-fungal tests were performed by sowing 1000 cells of *Candida albicans* on the previously prepared medium culture. Anti-fungal activity was determined by evaluating anti-fungal parameters values (minimal fungicidal concentrations [MFC] and IC50). **Results:** The results showed that all extracts possessed anti-fungal activities whose levels vary from plant species to another. Eight of them had a satisfactory anti-candidosic activity and extracts from *Terminalia* species were the most active. Among them the *Terminalia superba* extracts generated the strongest activities (MFC = 0.0975 mg/mL). Compared with ketoconazole (MFC = 0.390 mg/mL), the *T. superba* extracts, aqueous (MFC = 0.195 mg/mL) and hydroethanolic (0.0975 mg/mL) were successively twice and four times more active. The worst anti-fungal activity (MFC = 1600 mg/mL) was obtained with the *Guarea cedrata* aqueous extract. **Conclusion:** All medicinal plants extracts produced anti-fungal activities, and *T. superba* was the most active.

KEY WORDS: Anti-candidosic, anti-fungal, ketoconazole, plant extracts

INTRODUCTION

At the end of an ethnobotanical survey conducted in 1991 in the area of Issia (Cote d'Ivoire) several medicinal plants were collected and identified by our research team. These plants are commonly used in traditional circles by the healers for their curative virtues. A large number of plants among the cataloged species are granted anti-infectious properties [1]. The *in vitro* anti-microbial properties of some plants have already been assessed by some members of our research team. This study was initiated to verify the anti-microbial virtues which some plants are granted in order to continue the studies initiated by our team. In this report, we summarize the results obtained after some *in vitro* tests with aqueous and hydro-ethanolic extracts from each plant against *Candida albicans* one of the most pathogenic fungi species to human.

MATERIALS AND METHODS

Plant Material

Different parts of plants were collected in the area of Issia (Cote d'Ivoire) during the ethnobotanical investigations. The

collected parts were the steam barks of the trees species and the whole aerial parts for the herbaceous species. The studied plants species are the following:

- *Ceiba pentandra* (L.) Gaertn. (Harvested in Soubakaniédougou January 1, 1965, L. Aké-Assi Number 7601);
- *Entandrophragma angolense* (Welw.) C.D.C. (Côte d'Ivoire: Yakassé August 22, 1978, L. Aké-Assi Number 14198);
- *Entandrophragma cylindricum* (Sprague) (Mopri Forest, December 17, 1965, L. Aké-Assi Number 8348);
- *Guarea cedrata* (A. Chev.) Pellegr. (Côte d'Ivoire: National Parc of Azagny, January 22, 1986 L. Aké-Assi Number 17242);
- *Hunteria eburnea* Pichon (Abouabou forest, November 13, 1948, Mangenot and Aké-Assi Number 748);
- *Khaya ivorensis* A. Chev. (Côte d'Ivoire: Floristic National Center, Abidjan February 20, 1991, E. Aké-Assi Number 77);
- *Milicia excelsa* (Welw.) C.C. Berg (Agbo forest, September 25, 1957, Adjanohoun Number 1911B);
- *Mitracarpus villosus* (SW).DC. (Biankouma, August 11, 1975, L. Aké-Assi Number 12926);
- *Nesogordonia papaverifera* (A. Chev.) Cap. (Gabia, November 30, 1966, L. Aké-Assi Number 9320);
- *Physalis angulata* L. (Dabou, January 18, 1964, L. Aké Assi Number 7303);
- *Solenomostemon monostachyus* (P. Beauv.) Briq. (Man, November 15, 1953, L. Aké-Assi Number 2815);
- *Terminalia catappa* Linn. (Côte d'Ivoire: Azuretti, July 15, 1964, G. Cremers Number 266);
- *Terminalia ivorensis* A. Chev (Adiopodoumé, May 17, 1966, L. Aké-Assi Number 8855);
- *Terminalia mantaly* H. Perrier (Floristic National Center of Cocody University, Abidjan, May 23, 1995, E. Aké-Assi Number 217);
- *Terminalia superba* Engl. & Diels (Tonkoui Mountain February 26, 1969, L. Aké Assi Number 10477).

In the first step of the study some samples of all the plants species were identified and taxonomically authenticated by professor Aké-Assi at the Herbarium of the floristic national center of the Felix Houphouët-Boigny University (Abidjan, Côte d'Ivoire). In the second step, some great quantities of plant parts of each species were collected separately. The parts were carefully checked to take out all undesired specimen parts. The parts were then cut into small fragments and carefully air-dried for 2 weeks at ambient temperature in the laboratory, under continuous ventilation, away from sunlight and dust. After that step all, the vegetal pieces were crushed to a fine powder with an electrical grinder. Finally, the different types of powder were hermetically sealed in polyethylene bags and stored away from light and moisture until the time of extraction.

Extraction

Two sorts of extracts (aqueous and hydroethanolic) were prepared from the powder of each species. For the aqueous extract, 100 g of each type of powder were extracted in a solvent kept with 1 L of distilled water by homogenization in a blender. After six cycles of homogenization, the homogenates obtained were first wrung out in a fabric square and then filtered twice successively with absorbent cotton and once with Whatman 3 mm filter paper. The resulting filtrates were concentrated under vacuum using a Büchi rotary evaporator at 60°C [2]. Dark powder obtained is the aqueous crude extract. Hydroethanolic extracts were prepared following the same process by using a mixture of solvent ethanol 70% and water 30%.

Microorganism Studied

The tested fungi consist of a *C. albicans* (n 896/AB of 10.01.2000) clinical strain. It was both clinical isolate as well as authenticated and identified strain. This strain was provided by the Laboratory of Mycology of the Medical Sciences, Faculty of the Felix Houphouët-Boigny University (Abidjan Côte d'Ivoire).

C. albicans is an opportunistic fungus. It is a human normal communalist, and it belongs to the normal flora, but it becomes pathogenic when the immune system fails. It causes most of 83% of yeast infections [3,4]. The generalized infection caused by this fungus in individuals severely immuno-compromised often leads to death [5-9].

Anti-microbial Test

The anti-fungal activities were assessed by determining anti-fungal parameters values which are minimal fungicidal concentration (MFC); concentration that inhibits 99.99% of growth in the experimental tube compared with the growth control tube and concentration for 50% of inhibition (IC_{50}); graphically determined around each assay. For each extract, six replicate trials were conducted against *C. albicans* and ketoconazole was used as standards for anti-fungal assay. Ketoconazole was selected because it is an anti-fungal molecule usually prescribed to patients in the treatment of mycosis.

The anti-fungal tests were carried out on culture medium Sabouraud (Biomerieux, 51078 Ref: 777666501). The incorporation of plant extracts into the agar was made using the agar slanted double dilution method [2,10-12]. After this, all 10 tubes of each series were sterilized in an autoclave at 121°C for 15 min and then inclined with a small base at room temperature to allow their cooling and solidification of the agar. Both aqueous and hydroethanolic extracts were tested separately.

Fungal germs culture on slanted agar previously prepared was made by sowing 1000 cells of *C. albicans* [11]. These cultures were incubated at 30°C for 48 h. At the end of the incubation time, colonies were counted out by direct counting with a colony counter pen (Ceinceware, number 23382). The growth in the 10 experimental tubes was expressed as survival percentage, calculated, compared to 100% of growth in the growth control tube. The formula to calculate this is shown below. For the test tubes, concentrations of plant extracts ranged from 1600 to 0.024375 mg/mL with a ½ reason geometrical connection.

The processing of these data permitted to calculate the MFC values. It also made it possible to plot the curves of

activity of the extracts and the graphically determination of the IC_{50} values.

Formula to calculate the survival percentage:

$$S = \frac{n}{N} \times 100$$

S: Survival (%)
n: Number of colony in one experimental tube
N: Number of colony in the growth control tube.

Activity Classification Scale

The anti-fungal activities of the plant extracts were classified into the category following the scale below. The activity scale is divided into five levels on the basis of the MFC values.

Activity	Values
Very low activity	MFC values>50 mg/mL
Low activity	MFC value=50 mg/mL
Average activity	50 mg/mL>MFC values≥6.25 mg/mL
High activity	6.25 mg/mL>MFC values≥0.780 mg/mL
Very high activity	0.780 mg/mL>MFC values≥0.001526 mg/mL

RESULTS

The results were summarized in the form of curves of sensitivity and a table. The curves illustrate the evolution of the survival of *C. albicans* depending on the variation of the concentrations of the extracts [Figures 1 and 2]. In general, all sensitivity curves showed a progressively decreasing pace with slopes that are stronger or not as strong according to the extracts. The curves whose slopes are strong illustrate high anti-fungal potency and those that have a weak slope reveal low anti-fungal potency. They intersect the horizontal axis at different levels according to the extracts. The slopes of the curves of the extracts from the *Terminalia* species are the strongest. While the weakest slopes are observed with curve of extracts from *G. cedrata* (BOSS) and *E. cylindricum* (ECYL) [Figures 1 and 2]. The extracts from the other species have curves whose slopes are average. The decreasing shape of the activity curves shows that the 30 extracts acted according to a relation amount-effect.

Table 1 contains all the values of the anti-fungal parameters (MFC and the IC_{50}). All the extracts tested were active against *C. albicans*. The highest MFC values were produced by *G. cedrata*, while the lowest ones were produced by *T. superba*. All the hydroethanolic extracts from the four *Terminalia* species produced MFC values that are lower than the MFC value of ketoconazole.

In addition to the four *Terminalia* species, *E. angolense* (ETAN), *H. eburnea* (HUNT), *M. excelsa* (EMI) and *M. villosus* (MVS) are among those whose hydroethanolic extracts generated MFC values that are lowest than 50 mg/mL.

DISCUSSION

Analysis of the whole results shows that *C. albicans* are sensitive to all the extracts tested. The extracts were all capable of inhibiting the *in vitro* growth of the fungal germs. However, the levels of these anti-fungal activities are variable from one extract to another. As a matter of fact, the analysis of MFC values [Table 1] reveals that while some extracts resulted in very low MFC values, demonstrating their high efficiency, conversely extracts from some other species generated very high values of MFC revealing that they are not very efficient inhibiting the *C. albicans in vitro* growth. Among them the aqueous extract BOSS-X_{Aq} from *G. cedrata* (MFC = 1600 mg/mL) possesses the weakest anti-candidosic activity. On the other hand, the hydroethanolic extract TEKAM$_4$-X_0 from *T. superba*

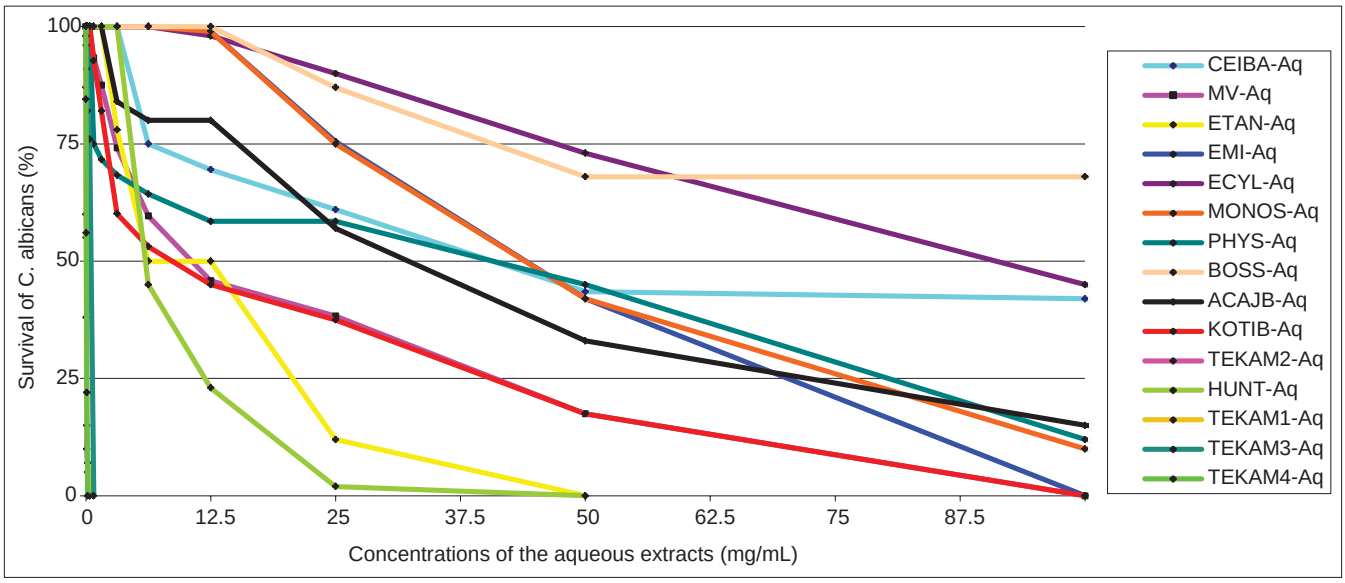

Figure 1: Antifungal activities of the crude extracts from 15 plants species. Sensitivity of *Candida albicans* to the aqueous extracts

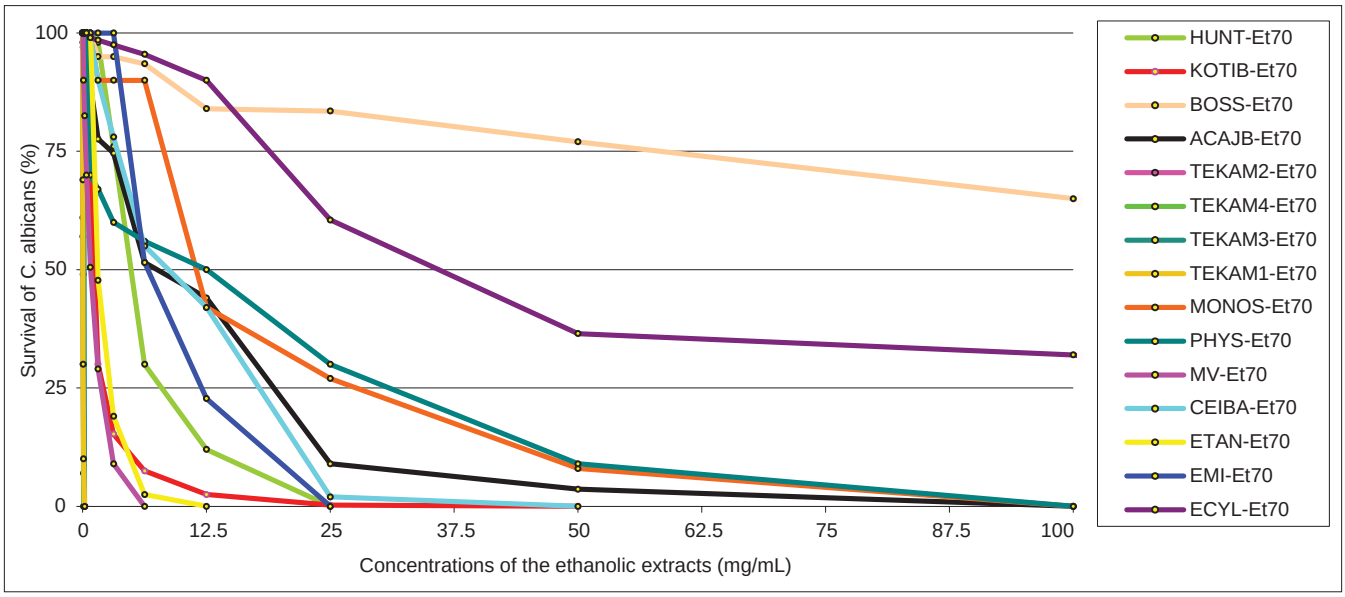

Figure 2: Sensitivity of *Candida albicans* to the hydroethanolic extracts

Table 1: Values of anti-fungal parameters of the extracts and reference anti-fungal drug

plants species and their code name	Values of anti-fungal parameters				Ratio of MFC values X_{Aq}/X_0 of each species
	IC_{50} (mg/mL)		MFC (mg/mL)		
	Aqueous extracts (X_{Aq})	Hydro-ethanolic extracts (X_0)	Aqueous extracts (X_{Aq})	Hydro-ethanolic extracts (X_0)	
C. pentandra (CEIBA)	40.625	8.571	200	50	4
E. angolense (ETAN)	6.25	1.524	50	12.5	4
E. cylindricum (ECYL)	91.071	36.071	400	200	2
G. cedrata (BOSS)	325.714	165	1600	800	2
H. eburnea (HUNT)	5.960	4.888	50	25	2
K. ivorensis (ACAJB)	8.705	7.589	200	100	2
M. excelsa (EMI)	8.705	6.563	100	25	4
M. villosus (MVS)	10.536	0.547	100	6.25	16
N. papaverifera (KOTIB)	8.705	1.181	200	50	4
P. angulata (PHYS)	40.179	12.5	200	100	2
S. monostachyus (MONOS)	43.75	11.473	200	100	2
T. catappa (TEKAM$_3$)	0.523	0.145	0.78	0.195	4
T. mantaly (TEKAM$_1$)	0.0423	0.03604	0.390	0.195	2
T. ivorensis (TEKAM$_2$)	0.054	0.0350	0.390	0.195	2
T. superba (TEKAM$_4$)	0.05746	0.03008	0.195	0.0975	2
Reference anti-fungal drug ketoconazole (KETO)	CI_{50} (mg/mL)		MFC (mg/mL)		
	0.01846		0.390		

IC_{50}: Inhibition concentration at 50%, MFC: Minimal fungicidal concentrations, *C. pentandra: Ceiba pentandra, E. angolense: Entandrophragma angolense, E. cylindricum: Entandrophragma cylindricum, G. cedrata: Guarea cedrata, H. eburnea: Hunteria eburnea, K. ivorensis: Khaya ivorensis, M. excelsa: Milicia excelsa, M. villosus: Mitracarpus villosus, N. papaverifera: Nesogordonia papaverifera, P. angulata: Physalis angulata, S. monostachyus: Solenomostemon monostachyus, T. catappa: Terminalia catappa, T. mantaly: Terminalia mantaly, T. ivorensis: Terminalia ivorensis, T. superba: Terminalia superba*

(CMF = 0.0975 mg/mL) exhibited the strongest anti-candidosic activity [Table 1].

On the basis of the classification scale of the levels of the activities, the analysis of the data shows that among the aqueous extracts, nine plant species produced extracts whose MFC values range from 100 mg/mL to 1600 mg/mL. This led to classify them as plants whose extracts possess some very low levels of anti-fungal activities. These species are the following: *C. pentandra*, *E. cylindricum*, *K. ivorensis*, *G. cedrata*, *M. excelsa*, *M. villosus*, *N. papaverifera*, *P. angulata*, *S. monostachyus*. For this reason, these species are not recommended to treat anti-fungal infections in traditional circles. Unless an extraction method permitting a better concentration of their active principles as well as the improvement of their anti-fungal activities was applied.

The results also show that the aqueous extracts from *E. angolense* and *H. eburnea* have an MFC value of 50 mg/mL so they have a low activity level (Table 1 and anti-fungal activity classification scale). In addition, the aqueous extract TEKAM$_3$-X_{Aq} from *T. catappa* inhibited the growth of *C. albicans* with a MFC value of 0.780 mg/mL. According to the anti-fungal activity classification scale, its anti-candidosic activity is classified as a high level of activity. Finally, the other three *Terminalia* species generated extracts whose MFC value are respectively 0.39 mg/mL for *T. ivorensis* and *T. mantaly*, 0.195 mg/mL for *T. superba*. This led to catalogue them in the category of those that have a very high level of anti-fungal activity (Table 1 and activity classification scale).

Otherwise, the comparison with previous works shows that the aqueous extract they got from *T. mantaly* was four times more active on *C. albicans* [13] than the same kind of extract we tested in this study. The MFC value obtained in their study was 0.0975 mg/mL for the aqueous extracts TEKAM$_1$-X_{Aq}, while the MFC value is 0.390 mg/mL in this present study for the same type of extract. On the other hand, the comparison with works on *T. superba* [14] reveals that TEKAM$_4$-X_{Aq} (MFC = 0.195 mg/mL) is two times more active than the

aqueous extract (MFC = 0.390 mg/mL) from *T. superba* tested by these authors. These differences of performance of these extracts could be explained by the fact that we did not collect the barks in the same area, and we did not test the extracts on the same *C. albicans* strain. The trees from which they collected the barks could contain low concentrations of active principles. And each fungal strain has its own sensitivity to anti-fungal drugs.

Meanwhile, the results from this study are in accordance with previous reports on the genus *Terminalia*, i.e. the works on *T. ivorensis* [15] and on *T. catappa* [16]. Because we obtained the same anti-fungal parameters values (MFC = 0.390 mg/mL and IC_{50} = 0.054 mg/mL for *T. ivorensis* and MFC = 0.780 mg/mL and IC_{50} = 0.523 mg/mL for *T. catappa*).

On the other hand, on the basis of MFC values, the comparison of the anti-fungal activities of all aqueous extracts shows that the aqueous extract from *T. superba* $TEKAM_4$-X_{Aq} (CMF = 0.195 mg/mL) is the most active of all. As a matter of fact, the MFC value ratios reveal that $TEKAM_4$-X_{Aq} is respectively two times more active than $TEKAM_1$-X_{Aq} (from *T. mantaly*) and $TEKAM_2$-X_{Aq} (*T. ivorensis*), it is also four times more active than $TEKAM_3$-X_{Aq} (*T. catappa*) and 256 times more active than ETAN-X_{Aq} (*E. angolense*) and HUNT-X_{Aq} (*H. eburnea*). In addition, the anti-candidosic activity of $TEKAM_4$-X_{Aq} is 512 times stronger than that of EMI-X_{Aq} (*M. excelsa*). $TEKAM_4$-X_{Aq} is also 1025 times more active than CEIBA-X_{Aq} (*C. pentandra*), ACAJB-X_{Aq} (*K. ivorensis*), PHYS-X_{Aq} (*P. angulata*), MONOS-X_{Aq} (*S. monostachyus*) and KOTIB-X_{Aq} (*N. papaverifera*). Lastly $TEKAM_4$-X_{Aq} is respectively 2051 times and 8205 times more active than ECYL-X_{Aq} (*E. cylindricum*) and BOSS-X_{Aq} (*G. cedrata*).

For the hydroethanolic extracts, the comparison of their performances on the basis of the MFC values highlights that for this kind of extracts, the four *Terminalia* species once more resulted in the lowest value of MFC [Table 1]. As a matter of fact, their MFC values range from 0.195 mg/mL to 0.0975 mg/mL [Table 1]. Among them, the hydroethanolic extract $TEKAM_4$-X_0 of *T. superba* possesses the lowest value of MFC (0.0975 mg/mL) meaning the strongest anti-candidosic activity. Inside this group of extracts possessing remarkable performances, $TEKAM_4$-X_0 is two times more active than $TEKAM_1$-X_0, $TEKAM_2$-X_0, and $TEKAM_3$-X_0. The last three extracts gave the same MFC value. However, the comparison of their IC_{50} values shows that $TEKAM_2$-X_0 (IC_{50} = 0.035 mg/mL) is the one that has the lowest IC_{50} value. So after $TEKAM_4$-X_0, $TEKAM_2$-X_0 is the second extract to be very active.

The results also show that four species (*E. angolense*, *H. eburnea*, *M. excelsa* and *M. villosus* produced hydroethanolic extracts whose MFC values range from 25 to 6.25 mg/mL [Table 1]. For this reason, their anti-candidosic activities are classified as the average level of activity. In this group, *M. villosus* is the species whose hydroethanolic extract is the most effective because it engendered the lowest MFC value (6.25 mg/mL).

Otherwise, from the comparison of the whole data of the hydroethanolic extracts the result is that BOSS-X_0 (*G. cedrata*) that produced the highest MFC value of 800 mg/mL, is the least active of all. In fact, it is 16,410 times less active than $TEKAM_4$-X_0 [Table 1]. So eventually, among all aqueous and hydroethanolic extracts, the extracts from *G. cedrata* produced the lowest anti-candidosic activities because their MFC values were the highest of all. On the other hand, extracts from the four *Terminalia* species were the most active; because they generated the lowest MFC and IC_{50} values against *C. albicans*. And particularly extracts from *T. superba* exhibited the best performances of all.

This strong anti-microbial potency of *T. superba* was also demonstrated by works on its methanolic extract which showed a broad spectrum of both anti-fungal and anti-bacterial activities [17]. This methanolic extract inhibited the *in vitro* growth of several strains of bacteria (*Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Citrobacter freundii*) and fungi (*C. albicans*, *Candida glabrata*, *Microsporum audouinii*, *Trichophyton rubrum*) [17].

Finally, the comparison of the performances of all extracts tested with that of ketoconazole, reveals that this reference anti-fungal drug is more active than most of extracts tested excepted few extracts from *Terminalia* species. As a matter of fact, KETO is 256 times more active than MONOS-X_0, PHYS-X_0 and ACAJB-X_0; 128 times more active than CEIBA-X_0 and KOTIB-X_0; 64 times more active than HUNT-X_0 and EMI-X_0; 32 times more active than ETAN-X_0; 16 times more active than MVS-X_0. Ketoconazole is also twice more active than $TEKAM_3$-X_{Aq}. On the other hand, KETO produced the same MFC value (0.390 mg/mL) as $TEKAM_1$-X_{Aq} and $TEKAM_2$-X_{Aq}. However, on the basis of their IC_{50} values, the comparison shows that Keto is respectively twice and three times more active than $TEKAM_1$-X_{Aq} and $TEKAM_2$-X_{Aq}.

However, compared with $TEKAM_1$-X_0, $TEKAM_2$-X_0, $TEKAM_3$-X_0 and $TEKAM_4$-X_{Aq} on the basis of their MFC values, KETO is respectively two times less active than these extracts. Finally, on the same basis of this comparison, $TEKAM_4$-X_0 (MFC = 0.0975 mg/mL), is clearly four times more active than ketoconazole [Table 1].

On the whole, the obtained data from this study highlighted the significant anti-fungal potency of plants of *Terminalia* genus. Otherwise, several investigations on plants of the genus *Terminalia* have already been conducted by some research teams. And many results of these previous reports share the results from the present study concerning their anti-microbial activities. Indeed, these works also confirmed the strong anti-microbial activities of extracts from a number of *Terminalia* species such as *T. catappa* [16,18,19], *T. chebula* [20], *T. glaucescens* [21], *T. ivorensis* [15,22-24], *T. macroptera* [25], *T. sericea* [26,27], and *T. superba* [14,17,28].

In addition, *M. villosus* was also reported for its broad spectrum of anti-fungal activities on *C. albicans*, *C. glabrata*, *C. tropicalis* and *A. fumigatus*, *A. flavus*, *C. neoformans*, *T. rubrum* and *T. mentagrophytes* [29-32]. The comparison reveals that on *C. albicans*, the *M. villosus* extracts [30] produced the same MFC values as the present report. However, on the basis of their IC_{50} values, they are two times less active than extracts from this study.

With other plant species, the comparison shows that $TEKAM_4$-X_{Aq} and $TEKAM_4$-X_0 of *T. superba* are respectively 256 times and 125 times more active than aqueous extract and hydroethanolic extract from *Microglossa pyrifolia* tested on *C. albicans* [2].

Moreover, the comparison of the whole data shows that for all plant species, hydroethanolic extracts are more active than the aqueous ones. As a matter of fact, for each case, hydroethanolic extracts produced MFC values that are 2-16 times lower than the MFC values of their aqueous equivalents [Table 1] if one establishes a comparison between extracts from the same plant species. This shows that for all plant species, hydro-ethanolic extracts concentrate a greater proportion of active principles than their aqueous equivalents. This assertion is similar to former investigation reports [2,13,15,16,29-31,33]. Indeed, the results of these previous investigations revealed that all the hydro-ethanolic extracts tested were 2-16 times more active than the equivalent aqueous extracts.

So we can deduce that between the two solvents used for extraction, the mixture ethanol-distilled water (70/30; v/v) is the solvent that permits the activity optimization and concentrates better the active principles from the different plants species. The active principles of all the plant species studied are more soluble in the mixture ethanol-distilled water (70/30; v/v). Considered under the chemical aspect, hydro-ethanolic extracts contain active principles that are of lipid nature and also polar molecules containing one or many oxygen atoms [2,34].

In addition, the active principles could be groups constituted by molecules of small sizes and average sizes (terpenoids, polyphenols, quinons, alkaloids, etc.) containing very low proportions of vegetal oil and chlorophyll [2,33]. Even if water can extract all type of molecules, water extracts contain a great content of macromolecules (polysaccharids, proteins and glycoproteins). They also contain a few species of polar lipids of small size, whose structures are simples. This high proportion of polysaccharids, glycoproteins and proteins could explain why aqueous extracts are always less active.

Alternatively as regards this aspect, the analysis of the antifungal parameter values of Table 1 shows that among the aqueous extracts, only four plant species (*T. catappa*, *T. ivorensis*, *T. mantaly*, *T. superba*) gave extracts exhibiting a high anti-fungal activity level. But for the hydro-ethanolic extracts, eight plant species (*E. angolense*, *H. eburnea*, *M. excelsa*, *M. villosus*, *T. catappa*, *T. ivorensis*, *T. mantaly*, *T. superba*) gave satisfactory anti-fungal activities.

Furthermore, many authors explained that most of the plants synthesize various secondary metabolites which are useful for their normal biology and to fight pathogenic microorganisms (virus, bacteria, fungi and various parasites) attacks [35-37]. These anti-microbial secondary metabolites are found melted among diverse substances and compounds extracted from plants. This would explain why the extracts from most of these plants have anti-fungal activities. The variability of their efficiency would be not only connected to various secondary metabolites content (alkaloids, terpenoids, polyphenols, quinons, coumarins) that plants produce, but also with the toxic power of these biomolecules to microorganisms [35,36,38].

CONCLUSION

This study showed that *C. albicans* are sensitive to all the extracts in dose-response relationship. All the 15 plant species produced active extracts with more or less raised performance levels. It proves and allows the understanding of the foundation of their use in traditional recipes, against infections. However, among them, only eight species gave extracts possessing good anti-fungal properties, allowing them to neutralize pathogenic microorganisms involved in infections. These species are *E. angolense*, *H. eburnea*, *M. excelsa*, *M. villosus*, *T. catappa*, *T. mantaly*, *T. ivorensis* and *T. superba*. But the most useful plants, because their extracts are by far very active, are the four *Terminalia* species (*T. catappa*, *T. ivorensis*, *T. mantaly*, and *T. superba*). The levels of their anti-fungal activities ranged from high to very high. Among them, the *T. superba* extracts generated the most excellent anti-candidosic activities. Furthermore, for each plant species, the hydroethanolic extracts were always noticeably 2-16 times as active as their equivalent aqueous.

Otherwise, the anti-fungal drug ketoconazole is clearly more active than most of extracts tested excepted $TEKAM_1$-X_0, $TEKAM_2$-X_0, $TEKAM_3$-X_0, $TEKAM_4$-X_{Aq} and $TEKAM_4$-X_0 the extracts from the *Terminalia* species. In fact $TEKAM_1$-X_0, $TEKAM_2$-X_0, $TEKAM_3$-X_0 and $TEKAM_4$-X_{Aq} are twice as active as ketoconazole; while $TEKAM_4$-X_0 is four times as active. This study confirms the real and strong anti-fungal activities of extracts of extract from the *Terminalia* species. Among the two solvents used for the extractions, the mixture ethanol-distilled water (70/30; v/v) is the solvent that permits the best optimization of the extraction and concentration of the active principles from a different plant species.

It would be thus desirable to continue this work by investigating more the anti-fungal potency of extracts from the four *Terminalia* species. Specifically our further works will aim the search and the isolation of compounds responsible for the anti-fungal activities as well as the determination of the chemical structure of these active principles. Our team hopes that these investigations will help to isolate new active principles that may be used in human therapeutic or to isolate metabolites that could serve as templates for synthesizing new and more active

molecules. These new molecules (natural or semi-synthetic) could then afford to expand the therapeutic arsenal and make it more effective.

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