

Comparative Study of the Anti-Candidal Activity of Four Medicinal Plants Used In Côte d'Ivoire

ABSTRACT

Several plants are used by traditional practitioners to treat infectious diseases. The ongoing development of antibiotic resistance continues to have a lasting impact on public health. Therefore, this study aimed to assess the potential offered by medicinal plants found in Ivorian soil. Spectrometric measurements, in addition to the moss test, revealed the presence of sterols and polyterpenes, polyphenolic compounds, saponins, quinones, alkaloids, and tannins in the extracts of the four plants studied. The use of the double dilution method in tilted tubes revealed the anti-candida potential of the plant extracts. The results obtained showed that the hydroethanolic extract of *T. mantaly* produced the lowest MFC (3.125 mg/mL) and IC₅₀ (0.3 mg/mL) values. It was followed by *Z. gilletii* (MFC = 3.125 mg/mL; IC₅₀ = 0.62 mg/mL) and *T. catappa* (MFC = 3.125 mg/mL; IC₅₀ = 0.68 mg/mL). No significant inhibition was observed with the extracts of *A. occidentale*. This study contributes to the valorization of medicinal plants and the floral heritage of Côte d'Ivoire.

Keywords: medicinal plants, extraction, anticandidosis activity.

1. INTRODUCTION

The yeast *Candida albicans* is primarily a human commensal that colonizes the mucosal surfaces of the gastrointestinal and genital tracts. However, *C. albicans* can, under certain circumstances, transition from commensalism to pathogenicity [1]. This transition is governed by fungal factors such as morphological changes, environmental factors like interactions with the intestinal microbiota, and the host's immune system.

Candida albicans has become a significant public health concern over the past two decades [2,3]. The spectrum of diseases caused by this species ranges from vaginal infections, affecting up to 75% of women at least once in their lifetime, to deep-seated infections in hospitalized patients, leading to high rates of morbidity and mortality. It may also play a role in the persistence or exacerbation of certain chronic inflammatory bowel diseases.

Candidiasis is highly recurrent, affecting between 138 and 140 million women worldwide every year [4,5], with 70-75% of them experiencing at least one episode of vulvovaginal candidiasis in their lifetime [6,7]. Incidence has doubled over the past 20 years, unlike gonococcal and *Trichomonas vaginitis*, which have seen a parallel decrease [8]. *C. albicans* remains the most common species (77-95%), followed by non-albicans *Candida* (20-30%), with the most common being *C. glabrata*[9].

The prevalence of candidiasis varies from one country to another. It was 35.52% in Cameroon [10], 32.6% in Senegal [11], and 38.9% in Benin [12]. In Côte d'Ivoire, *Candida albicans* is responsible for 16.3% of otomycoses [13], 59.6% of

vaginal candidiasis [14], and is found in 13.5% of HIV-positive patients suffering from chronic diarrhea. Consequently, various herbal preparations are recommended in the market to treat candidiasis.

The resistance of some commercially used antifungal agents has made it necessary to seek naturally effective alternatives. Plants have been used in traditional phytotherapy for years. In some parts of the world, plants and herbs are still the primary sources of remedies used in disease treatment [15]. Additionally, in low- and middle-income countries, approximately 80% of people rely on medicinal plants for their primary healthcare needs [16,17]. In order to contribute to the management of candidosis infections, this study aims to evaluate the antifungal activity of four plants species on the *in vitro* growth of *C. albicans*.

2. MATERIAL AND METHODS

The plant material used consists of various parts of four different plants (Table 1), collected separately. These various plant organs were harvested in Côte d'Ivoire. A herbarium was created for each plant to identify the different plants collected at the National Center Floristics (CNF).

The *C. albicans* isolates were provided to us by the mycology laboratory of the Pharmacodynamic Biochemical Research and Teaching Unit. The choice of this clinical strain was motivated by its confirmed pathogenicity in humans and its resistance to Fluconazole, Voriconazole, and Itraconazole.

Table 1: plant material used

Plante	Part use	Place of harvest	Morphology
<i>Anarcadium occidentale</i> L. (Anacardiaceae)		Abengourou	Tree
<i>Terminalia catappa</i> L.	Trunk bark	Agboville	Tree
<i>Terminalia mantaly</i>		Agboville	Tree
<i>Zanthoxylum giletii</i>		Abengourou	Tree

2.1 PLANT COLLECTION AND PACKAGING

The organs of each plant species (individually) were harvested in large quantities and transported to the biochemistry laboratory for sorting. For each sample of a specific species, foreign specimens were removed, and the harvests were cleaned of any samples contaminated by pesticides or other agricultural products, rotten, burned by fires, or infested with mold or other parasites.

Except for the bark, the other plant components were rinsed, drained by shaking, and cut into small pieces. These plant pieces were then dried away from direct sunlight at room temperature (25-30°C) for three weeks. The drying process was carried out in a well-ventilated area. After drying, the plant components (for each plant species taken separately) were finely ground using an IKA-Labortechnik electric grinder (Type MFC/Janke & Kunkel). The different powders were packaged separately in non-translucent glass jars. These containers were then sealed, labeled, and stored (in the laboratory, under a hood) away from moisture and heat. The obtained powders were stored for later use.

2.2 EXTRACTION METHOD

Aqueous and hydroethanolic extracts were prepared following the method of Zirih[18], using distilled water as a solvent and an ethanol-water mixture (70/30, v/v). To do this, one hundred grams (100 g) of plant powder were dissolved in one liter of solvent, resulting in a 1:10 (w/v) ratio. The mixture was vigorously homogenized using a blender. The homogenate was squeezed through a percale cloth and then successively filtered three times using hydrophilic cotton and filter paper. The filtrate was evaporated at 45°C using a Venticell® ventilated drying oven for 24 hours for the 70% ethanol extract and 48 to 72 hours for the aqueous extract[19].

2.3 Phytochemical sorting

Phytochemical sorting is a method for characterizing the main chemical groups, such as sterols, polyterpenes, alkaloids, tannins, phenolic compounds, flavonoids, quinones and saponins. These compounds were highlighted using appropriate reagents that reacted with chemical compounds to give specific colors or precipitates that would attest to the presence or absence of the desired molecules in the extracts. This study was carried out with the total extracts (aqueous and hydroethanolic) of each plant, following the protocol of Békro[20].

2.4 DETERMINATION OF ANTIFUNGAL ACTIVITY OF EXTRACTS

2.4.1 Preparation of Agar

The amount of agar needed for the test was prepared according to the supplier's instructions. Forty-two grams (42 g) of powder were homogenized in 1 L of distilled water. The mixture was stirred and heated on an IKA MAG hotplate for 5 minutes until complete homogeneity was achieved.

2.4.2 Incorporation of Extract into Agar

Tests were conducted separately for each extract and for each fungal species in order to determine antifungal parameter values. The comparison of these parameters would lead to the selection of the most active plant extract. The double dilution method in slanted tubes was used to incorporate the extracts into the agar. Precooked agar was poured into 10 test tubes numbered from 1 to 10, with 20 mL in tube No. 1 and 10 mL in the other tubes (No. 2 to No. 10) in each series. Among these 10 tubes, 8 were test tubes containing the plant extract, and 2 were control tubes without plant extract: one serving as a control for germ growth (GC), and the other without germs serving as a control for culture medium sterility (SC). Overall, and according to the test series, concentrations ranged from 1000 µg/mL to 0.38 µg/mL. For the 8 test tubes in each series, concentrations varied geometrically by a factor of ½, from tube No. 1 to tube No. 8. After incorporating the extract, all 10 tubes in each series were sterilized in an autoclave at 121°C for 15 minutes and then slanted with a small base at room temperature to allow cooling and agar solidification.

2.4.3 Inoculum Preparation

The inoculum was prepared from young cultures of *C. albicans*, which were 48 hours old. A well-isolated colony was taken with a loop and homogenized in 10 mL of sterile distilled water. This resulted in the 10⁰ concentrated mother suspension of 10⁶ cells/mL. From this suspension, a 10⁻¹ suspension was prepared by diluting the first suspension 1:10 by transferring 1 mL of the suspension into 9 mL of sterile distilled water. This final suspension contained 10⁵ cells/mL.

2.4.4 Tube Inoculation

For each test tube in each series, except for the SC tube, *C. albicans* culture was performed on the agar media prepared earlier by streaking 10 µL of the 10⁻¹ suspension until the entire droplet was exhausted. The spreading was done over the entire surface of the agar. These 10 µL corresponded to 1000 cells. The cultures thus prepared were incubated in an incubator at 30°C for 72 hours. The tests were conducted under strict sterile conditions. The experiments were repeated 5 times for each plant extract[19,21].

2.4.5 Counting of Colonies

At the end of the incubation period, the colonies of each fungal species were counted by direct counting using a colony counter. Subsequently, the growth in the 8 experimental tubes of each series was evaluated as a percentage of survival calculated relative to 100% survival in the control growth tube. The percentage of survival of fungal germs in the experimental tubes was expressed by the formula:

S: Germ survival (expressed as a percentage)

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

N: Number of colonies in growth control tube

n : number of colonies in experimental tube

Processing of the experimental data enabled us to plot the survival curves that helped determine the antifungal parameter values. These are:

- Minimum Fungicidal Concentration (MFC): this is the lowest concentration of extract in the tube that gives 99.99% inhibition compared with the growth control tube. Conversely, it is the concentration that gives 0.01% survival compared with the growth control tube.

- Concentration for 50% Inhibition (CI50): this is the concentration giving 50% estimated inhibition in relation to the number of colonies counted in the growth control tube. This parameter is determined graphically from the survival curve[18].

3. RESULTS

3.1 EXTRACTION YIELDS

The different extraction yields are shown in figure 1 below. Aqueous extracts gave the highest yields, with values ranging from 27.12 to 19.27%. Hydroethanol extracts generated values ranging from 12.18 to 20.14%. The best aqueous yield was obtained with *T. catappa* and the best hydroethanolic yield was obtained with *T. mantaly*.

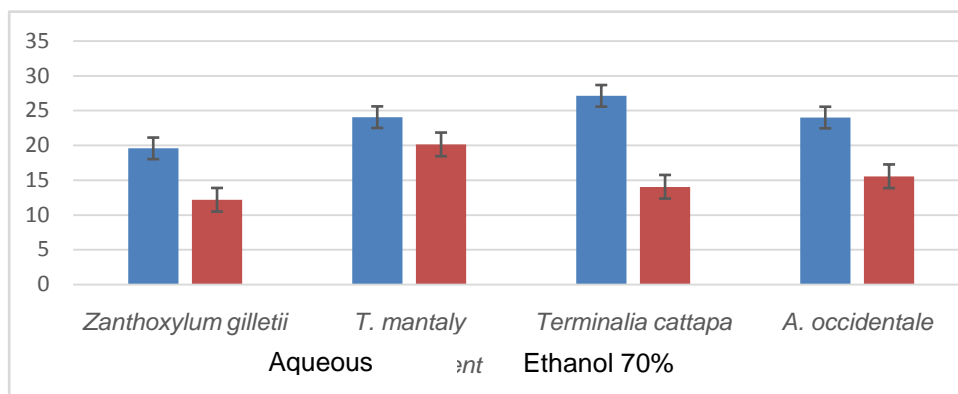


Figure 1: total extract yield

3.2 PHYTOCHEMICAL STUDY

The screening allowed us to identify the main chemical groups present in the various extracts (Table 2). The plus sign indicates the presence of the metabolite, while the minus sign indicates its absence. Thus, we found all the targeted groups in the aqueous extracts of each plant, with the exception of saponins; the hydroethanolic extracts contained the other six secondary metabolites we were searching for.

Table 2: Chemical groups present in the total extracts of the four plant species

Plants	Extracts	Chemical groups						
		Sterols and polyterpenes	Phenolic compounds	Flavonoids	Tanins	Quinones	Alkaloids	Saponosides
<i>Anarcadium occidentale</i>	aqueous	+	+	+	+	+	+	+
	Ethanol 70%	+	+	+	+	+	+	-
<i>Terminalia catappa</i>	aqueux	+	+	+	+	+	+	+
	Ethanol 70%	+	+	+	+	+	+	-
<i>Terminalia mentaly</i>	aqueux	+	+	+	+	+	+	+
	Ethanol 70%	+	+	+	+	+	+	-
<i>Zanthoxylum gillettii</i>	aqueux	+	+	+	+	+	+	+
	Ethanol 70%	+	+	+	+	+	+	-

3.3 ANTICANDIDAL ACTIVITY OF PLANT EXTRACTS

The results reveal that the four medicinal plants have different activities. The parameter values obtained for the aqueous and hydroethanolic extracts are summarised in Table 3.

Table 3. Aqueous and hydroethanolic extracts are summarised

Plants		<i>Candida albicans</i>	
		Antifungal parameters (mg/mL)	
		IC50	MFC
<i>Anarcadium occidentale</i>	aqueous	ND	ND
	Ethanol 70%	ND	ND
<i>Terminalia catappa</i>	aqueous	1,19	12,5
	Ethanol 70%	0,68	3,125
<i>Terminalia mentaly</i>	aqueous	0,35	6,25
	Ethanol 70%	0,3	3,125

Zanthoxylum gilletii	aqueous	1,1	12,5
	Ethanol 70%	0,62	3,125

4. DISCUSSION

The antifungal properties of four plants used in traditional medicine were studied. Extractions were carried out using distilled water and 70% ethanol on various plant parts. The extractions in this study were performed under similar cold conditions and for a relatively equal duration. The data shows that, overall, the yields of aqueous extractions range from 27.12% to 19.57%, while those of hydroethanolic extractions range from 20.14% to 12.18%. A comparison reveals that the best yields were obtained with distilled water, which can be attributed to the polarity of distilled water. These results differ from those of Kra [21], who found that hydroalcoholic extraction yielded better results.

To determine the nature of the chemical groups present in the extracts, phytochemical screening was conducted on the plant-derived extracts. Secondary metabolites in medicinal plants form the material basis of their clinically curative effects and are important indicators to assess the quality of medicinal materials. Therefore, it is necessary to identify them in the obtained extracts.

The data obtained reveal the presence of Sterols and Polyterpenes, Phenolic Compounds, Flavonoids, Tannins, Quinones, Alkaloids, and Saponins in the aqueous extracts of all the plants. Except for Saponins, these metabolites were also found in the hydroethanolic extracts. The results obtained with *A. occidentale* are consistent with those obtained by Kikakedimau [22]. The metabolites found in *Z. gilletii* by Abiodun [23] are the same as those found in this study. Regarding the *Terminalia* species, the results of the phytochemical screening of *T. cattapa* and *T. mantaly* are identical to the data from the works of Yayé [24] and Bérenger [25], respectively. In addition to Sterols, Polyterpenes, Phenolic Compounds, Quinones, Alkaloids, and Saponins, Bérenger [25] also found other compounds such as protoanthocyanins and anthraquinones. Abiodun [23] also found leucoanthocyanins and cardiac glycosides, which were not investigated in our study.

The presence of these phytochemical substances in various parts of these four plants could justify their use in the treatment of microbial infections mentioned in the literature.

Regarding antifungal activity, the analysis of the data on the evaluation of the anticandidal activity of the eight total extracts from four plants reveals that the hydroethanolic extracts exhibited better antifungal activities than the aqueous extracts from all the plants. The antifungal test data shows that, for each plant, the hydroethanolic extracts generated lower MFC values than their aqueous counterparts during the tests. These results are similar to those of Yayé [24], Ouattara [26], Bagré [27]. According to these authors, this superior performance of the hydroethanolic extracts is attributed to their higher qualitative richness in secondary metabolites (SM).

The anticandidal activity carried out with the total extracts of *A. occidentale* did not show any confirmed activity. In fact, the colony counts in the various experimental tubes indicated a reduction in candidal load compared to the growth control tubes. Therefore, no antifungal parameters (IC₅₀ and MFC) could be determined. This lack of clear activity could be due to the resistance profile of the *C. albicans* species used. The absence of activity in *A. occidentale* in this study does not refute its traditional use. Indeed, a study conducted by Batista [28] on several bacterial species resulted in MFC values ranging from 50 to 200 mg/mL. This study justifies the traditional use of this plant in the case of infectious diseases.

The analysis of the activities of the extracts from *Z. gilletii*, *T. cattapa*, and *T. mantaly* corroborates their traditional use. Indeed, all these extracts showed MIC values. The values obtained with the hydroethanolic extracts were lower than those generated by the aqueous extracts. Based on the MFC values, the hydroethanolic extracts of each plant were at least two times lower than their aqueous equivalents. From the comparative analysis of the extracts from different plants, it is evident that *T. mantaly* has more active extracts than those of *T. cattapa* and *Z. gilletii*. The MFC values generated by *T. cattapa* and *Z. gilletii* are identical for some extracts. When comparing IC₅₀ values, it is evident that *Z. gilletii* is more active than *T. cattapa*.

The parameters obtained from the aqueous and hydroethanolic extracts of *Z. gilletii*, *T. cattapa*, and *T. mantaly* align with the interest of traditional medicine in the treatment of infections. These values also confirm the presence of terpenoid, phenolic, and alkaloid metabolites with antimicrobial activity.

4. CONCLUSION

This study aimed to analyze the anticandidal potential of hydroalcoholic and aqueous extracts from four plants widely used in African pharmacopoeia. The hydroethanolic extract of *T. mantaly* showed the highest activity against the multi-resistant strain of *C. albicans* tested. The extracts of *Z. gilletii* and *T. cattapa* also exhibited inhibitory effects on *C. albicans*. A marked lack of activity was observed with *A. occidentale* against this multi-resistant yeast. The level of activity observed with these extracts suggests that medicinal plants have an inhibitory effect on *C. albicans*. However, the use of medicinal plants should be regulated, considering the results obtained with the extracts of *A. occidentale* and other factors. This study contributes to the valorization of medicinal plants and the floral heritage of Côte d'Ivoire.

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