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Anticandidosic activity of selected medicinal plants from Côte d'Ivoire

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Superficial candidiasis is a real public health problem, especially in immunocompromised people and HIV-infected patients. Due to the toxic handicap and the high cost of antifungal drug treatment, people use medicinal plants, which are relatively more accessible. Medicinal plants are an important source of novel antimicrobial agents. This study aims to determine the *in vitro* antimicrobial activity of aqueous and methanolic extracts from different parts of plants species against *Candida albicans*. Six plants species from the flora of Côte d'Ivoire were screened for anticandidal activities by ethnobotanical study. These plants species were: *Bridelia ferruginea*, *Citrus aurantium, Pycnanthus angolensis, Desmodium adscendens, Zanthoxylum zanthoxyloides Lam.* and *Mareya micrantha L.* Methanolic and aqueous extracts from the powder of leaves and/or barks samples of each species were tested using the bioautographic method in F254 glass silica gel plate, with Miconazole as reference. Bioautography assay was used to account antifungal compounds in plant extracts. *Bridelia ferruginea*, *Pycnanthus angolensis,* and *Zanthoxylum zanthoxyloides* Lam showed good activity. The minimal inhibitory concentrations (MIC) values against *Candida albicans* ranged between 12.5 and 100 mg/ml. Bioautography results demonstrated that active chemical compounds of the alcoholic extracts of the plants (flavonoids, alcaloids, polyterpenes, polyphenols and sterols) were responsible for antimicrobial activity. The Ivorian pharmacopoeia is full of medicinal remedies. Three plants of our study have shown a good activity on strains of *Candida albicans*.

Key words: *Candida albicans*, antifungal activity, plants, bioautography.

INTRODUCTION

Candida yeast genus is the most common opportunistic pathogen among fungal infections causing high mortality and morbidity. It involves yeast pathogenic isolated from skin, mouth, intestinal tract, and vagina. Over the past few decades, the incidence of candidiasis has increased especially with the growing number of immunocompromised patients (Essid et al., 2017). Resistance of *Candida* yeast genus to current medications, including the azoles, was reported (Arendrup, 2013). The search for new alternative

strategies is therefore important in order to fight *Candida* infections. Phytotherapy represents a more effective and less toxic alternative than standard drugs as anti-candida agents (Alves et al., 2014; Teodoro et al., 2015). Traditional medicine (TM) is often the primary mode of health care used by population in many countries due to poverty. It is an important and often underestimated part of health care. More than 80% of the population in Africa use TM to serve their health needs, according to the World Health Organisation (Anon n.d.). Plants are a primary source of new natural medicinal products. These traditional remedies use any part of plant, such as barks, leaves, flowers, roots and seeds. These remedies can be prepared from a single plant or a combination of different plants. Medicinal plants contain bioactive compounds with healing ability. These include saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds found as secondary metabolites in plants (Mahlo et al., 2016). Africa medicinal flora is rich and medicinal plant knowledge nowadays has a great development espacilly in Côte d'Ivoire. The country benefits from an excellent floristic biodiversity and the indigenous people have old knowledge of the medicinal plant uses. Antimicrobial activities of extracts from *Bridelia ferruginea* (Afolayan et al., 2018; Alowanou et al., 2015), *Citrus aurantium* (Metoui et al., 2015; Ruiz-Pérez et al., 2016), *Pycnanthus angolensis* (Kuete et al., 2011), *Zanthoxylum zanthoxyloides Lam.* (Tine et al., 2017) and many other herbs have also been reported. Indeed, Kra Mathieu et al. showed that the hydroethanolic extracts from the four *Terminalia* species produced minimal fungicidal concentrations (MFC) values that are lower than the MFC value of ketoconazole (Mathieu et al., 2014). Other plant species like *Borreria latifolia, Borreria verticillata, Erigeron floribundus, Euphorbia hirta, Turraea heterophylla and Vernonia colorata* were tested (Bi et al., 2007).

In this study, we chose six Ivoirian plants species used by traditional healers for the treatment of superficial candidiasis: *Bridelia ferruginea, Citrus aurantium, Pycnanthus angolensis, Desmodium adscendens, Zanthoxylum zanthoxyloides Lam. and Mareya micrantha L.* These plants have never been evaluated in Côte d'Ivoire for their antifungal properties. We investigated their aqueous and methanolic extracts.

MATERIALS AND METHODS

Plants collection

For the identification of medicinal plants used to treat superficial candidiasis, we bought them from sellers in different markets of Abidjan (Abobo, Adjamé and Treichville). These markets are located in the popular neighborhoods of the city of Abidjan. Different plants and recipes were identified from these sellers according to their skills. The formulas were combinations of plants used together. Then, the National Center of Floristry in Abidjan located at the University Félix Houphouët Boigny helped us to identify correctly all the plants bought in the markets. The selected plants for this study met the following criteria: (i) used by population in the treatment of superficial candidiasis; (ii) existed in the African pharmacopoeia; (iii) widespread in Côte d'Ivoire and easily accessible. Thus, six plants species from traditional medicine were selected namely: *B. ferruginea*, *C. aurantium*, *P. angolensis, D. adscendens, Z. zanthoxyloides* Lam. and *M. micrantha L.* The plant materials were the bark of *Pycnanthus angolensis*, the creeping stems of *Desmodium adscendens*, the roots of *Bridelia ferruginea* and the leaves of *C. aurantium*, *Z. zanthoxyloides* Lam. and *M. micrantha L.*

The plant materials were harvested in three parts of Côte d'Ivoire:

1. In the Banco forest, precisely on the slope overlooking the district "Anokoua" in the city of Abobo southern Abidjan (*D. adscendens*, *C. aurantium*, *P. angolensis* and *M. micrantha L.*),
2. in the city of Toumodi in central Côte d'Ivoire (*B. ferruginea*)
3. in the city of Korhogo in Northern Côte d'Ivoire (*Z. zanthoxyloides Lam*).

The plants materials were dried at room temperature (25°C) for about 8 weeks and then ground to fine powder. It was stored in airtight bottles in the dark until extraction to prevent oxidation.

Plant extraction

Preparation of total extracts of drugs

Each finely ground plant material (60 g) was extracted with 500 ml of solvents in erlenmeyer: water or methanol. Extracts (total aqueous, ethanolic 70% and residual extracts aqueous) were prepared according to the method described by Camara et al. (2016). Plant extracts were re-dissolved in acetone for microbiological assays and phytochemical analysis.

Phytochemical analysis

Colorimetric and precipitation reactions were used for phytochemical sorting; they highlight the following chemical groups: sterols and polyterpenes, polyphenols, flavonoids, tannins, quinonic substances and alkaloids. Chemical components of each extracts were analysed and used to determine their anticandidal activity.

C. albicans strains

Clinical strains were obtained from the parasitology and mycology laboratory of CeDReS. The germ tube test and the chlamydospore formation assay were used for identification of *C. albicans* yeast by

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Table 1. Parts of plants and their uses.

Plants	Familly	Used parts	Form of use
Bridelia ferruginea	Euphorbiaceae	Roots	Maceration, decoction
Citrus aurantium	Rutaceae	Leafs, fruits	Cataplasm, maceration, decoction
Desmodium adscendens	Fabaceae	Leafs, Roots bark	Decoction, toothbrush
Pycnanthus angolensis	Myristicaceae	Stem bark	Decoction, cataplasm
Mareya micrantha	Euphorbiaceae	Leafs	Decoction, cataplasm
Zanthoxylum zanthoxyloides	Rutaceae	Leafs	Decoction, toothbrush

using young colonies (24 h).

Anticandidosic activity assay: Bioautography

A young *C. albicans* colony was collected and homogenized in 9 mL of sterile distilled water. The first inoculum was obtained by mixing 0.5 mL of the yeast suspension to 25 mL of Sabouraud medium liquid incubated at 30°C for 15 h. The second inoculum was obtained by mixing 0.5 ml of the first one to 25 mL of Sabouraud medium liquid incubated at 30°C for 8 h. Both incubations (15 and 8 h) allowed the yeasts to be in exponential growth phase.

In the next step, plant extracts (100 mg) were serially diluted at 50% with chloroform (1 mL). Thifty microliters of each dilution (S1, S2, S3, and S4) of the total plant extracts and 50 µLof the control (Miconazol: T) are put on a silica plate. The deposits are then quickly dried with an electric dryer. Sabouraud maltose agar medium was regenerated at 40°C and was mixed with the inoculum at the proportion of 0.5 mL of inoculum in 25 mL of malted Sabouraud medium. Then, 20 mL of this mixture was poured on the silica plate G bearing the deposits of the serial extracts dilutions (alcoholic and aqueous). This step was performed on a heating plate to avoid solidification of the cast medium. The silica plate was incubated in the wet chamber at 30° for 24 h. After this time, the silica plate G was removed from the oven and sprayed with 4 mL of thiazolyl blue solution (2.5 mg/mL of thiazolyl blue in sterile distilled water). At the end, this silica gel plate was incubated again for 4 h in the wet chamber at 30°C. The reading was made with revelatory. The minimal inhibit concentration (MIC) was recorded as the lowest concentration of the extract that inhibited antifungal growth.

The antifungal test of each compound (alkaloids, polyterpenes, sterols and flavonoids) was carried out in the same way as the anticandidotic test on total plant extracts. These tests were performed three times to confirm the results.

RESULTS

The ethnobotanical study of plants with presumed activity on *C. albicans* for medicinal plant sellers identified 20 plants. Overall, medicinal plants are used in combination with other plants for the same treatment. We therefore selected those that returned in the majority of the preparations. In the current study, six medicinal plants were investigated for their antifungal activities against *C. albicans*. Table 1 shows the parts of the plants used and their use. The commonly methods used remain decoction, which consists of introducing plant parts into boiling water.

Phytochemical sorting allowed extraction of chemical groups (sterols and polyterpenes, polyphenols, flavonoids, tannins, quinonic substances and alkaloids). Table 2 shows the chemical composition of the aqueous and alcoholic extracts. Sterols and polyterpenes, polyphenols, flavonoids and alkaloids were mainly found.

In the present study, the extracts of six medicinal plants were investigated for their antifungal activities against *C. albicans.* The details of the results are shown in Table 3. *C. albicans* strains are susceptible to alcoholic extract of three plants (*Bridelia ferruginea*, *Pycnanthus angolensis* and *Z. zanthoxyloides Lam*. The minimal inhibition concentration (MIC) values against *C. albicans* were 12.5 mg/ml for the three plants. This activity seems to be the result of chemical compounds extracted during phytochemical sorting. Determination of the active chemical compounds of the alcoholic extract showed that total flavonoid of *B. ferruginea,* total quinones of *P. angolensis* and total alkaloids of *Z. zanthoxyloides* Lam. gave an inhibition zone similar to the Miconazole one.

DISCUSSION

C. albicans infections are widespread. Under normal conditions, this fungus is harmless and found in the intestinal walls, mouth, vagina and skin. If the natural balance is upset, depending on the soil on which the *Candida* proliferates, it could cause superficial

Table 2. Phytochemical constituents.

Plants	Extract	Sterols / Polyterpenes	Polyphenols	Flavonoids	Tanins		Quinonics Substances	Alkaloids	
					Gal	Cat		D	B
Bridelia ferruginea	H2O	+	+	-	-	-	-	+	+
	OH	+	+	+	-	-	-	+	+
Citrus aurantium	H2O	+	+	-	-	-	-	+	+
	OH	+	+	+	-	-	-	+	+
Mareya micrantha L	H2O	-	+	-	-	+	-	-	-
	OH	-	+	-	+	+	-	-	-
Desmodium adscendens	H2O	+	+	+	-	-	-	+	+
	OH	+	+	+	-	-	-	+	+
Pycnanthus angolensis	H2O	-	-	+	-	-	-	+	+
	OH	+	+	+	-	-	+	+	+
Zanthoxylum zanthoxyloides	H2O	-	+	+	-	-	-	-	-
	OH	-	+	+	-	-	-	+	+

+, positive reaction (presence of the searched chemical group); -, negative reaction (absence of the searched chemical group); gal, gallic acid; cat, catechin; D, Dragendorf; B, Bouchardat.

Table 3. Plants with anticandidal activity (*Bridelia ferruginea*, *Pycnanthus angolensis,* and *Zanthoxylum zanthoxyloides* Lam.).

Plants	Extract	Concentrations (mg/l)	Inhibition zone (mm)
Bridelia ferruginea	H_2O	100	0
		12.5	0
	OH	100	13 ± 2
		12.5	11 ± 2
Pycnanthus angolensis	H_2O	100	0
		12.5	0
	OH	100	13 ± 5
		12.5	11 ± 2
Zanthoxylum zanthoxyloides	H_2O	100	0
		12.5	0
	OH	100	13 ± 2
		12.5	11 ± 2
Miconazole		100	11± 4

candidiasis (cutaneous or mucous membrane) or deep candidiasis. Faced with this pathology, traditional medicine therapeutic is usually used in our regions. The current study aims to evaluate the anticandidal effectiveness of six plants and their main components. *Bridelia ferruginea*, *Citrus aurantium*, *Pycnanthus angolensis, Desmodium adscendens, Zanthoxylum zanthoxyloides Lam.* and *Mareya micrantha L.* are from African particularly Ivorian pharmacopoeia and their antifungal activities are known. *Bridelia ferruginea* for example is used for antiamebic, antianemic, antibacterial, anticonvulsant, anti-diabetic, antidiarrhoeal, antihelmintic, anti-inflammatory, antimalarial, antinociceptive, antiviral, hypoglycemic and for abdominal pain (Awodele et al., 2015; Ngueyem et al., 2009). *Pycnanthus angolensis* showed antimalarial, antibacterial, hypoglycemic and anticancer activities (Fort et al., 2000; Gbolade and Adeyemi, 2008; Abrantes et al., 2008). *Z. zanthoxyloides Lam.* also has an action on hematological parameters and oxidation (Ogunbolude et al., 2014; Ikumawoyi et al., 2016). Decoction is the most commonly used method of preparation and is obtained by boiling plants in water. Otherwise, this method according to the stakeholders surveyed would be able to use all the plants' potential.

Most traditional healers use water for their decoctions because water is the only solvent available. A major challenge of using water for extraction is that non-polar bioactive compounds cannot be extracted. The type of solvent used in the extraction procedure determines the success of isolating compounds from plant material (Masoko and Nxumalo, 2013). We used two types of solvents in this study: water and methanol. The phytochemical composition revealed that both solvents were able to extract the same chemical compounds from the plants studied. However, in our study, the tests from extracts for antifungal activities against *C. albicans* showed that only alcoholic extracts were active. Other studies reported that alcohols, particularly methanol, allowed the best extraction of active substances from plants (Arama et al., 2016; Masoko and Makgapeetja, 2015).

The bioautography assay was used to determine antifungal activity. This study highlighted the anti-*Candida* potency of three from the six studied plants (*Bridelia ferruginea*, *Pycnanthus angolensis* and *Z. zanthoxyloides Lam.*). Many studies showed that *Bridelia ferruginea*, *Pycnanthus angolensis* and *Zanthoxylum zanthoxyloides Lam.* have antimicrobial activities in particular antifungal effect (Afolayan et al., 2018; Alowanou et al., 2015; Kuete et al., 2011; Tine et al., 2017). Besides, chemical groups from these three anti-*Candida* plants contained flavonoids quinones and alkaloids responsible for this activity. The antifungal activity of these compounds has been reported in the literature. Similarly, many authors tested the anticandidotic activity of flavonoids (Seleem et al., 2017; Ozçelik et al., 2006; Orhan et al., 2010). Kamdem Wabo et al. tested the anticandidotic activity of a new quinones as Pycnanthuquinone C isolated from *Pycnanthus angolensis* (Wabo et al., 2007). Alkaloids have also been reported to possess antifungal activity (Orhana et al., 2007). The remaining three plant species (*Citrus aurantium*, *Desmodium adscendens,* and *Mareya micrantha L.*) showed no activity in the bioautography screening against *C. albicans*. This could be explained by the fact that some of the active compounds were volatile and evaporated during drying period prior to bioautography. Biological activity synergism between different compounds in the extracts is also a possible reason (Mahlo et al., 2016).

The results presented in this study are encouraging. A wider study is needed to identify the effective components, mode of action and possible toxic effect *in vivo* of these ingredients. It is therefore important to investigate the potential of these plants as novel antifungal agents, targeting the multidrug resistant fungi of clinical importance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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