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ARTICLE

Anticandidosic activity of selected medicinal plants from Côte d’Ivoire
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Akoua Valérie Bédia-Tanoh, Sébastien Miézan, Pulchérie Kiki-Barro,
Kondo Fulgence Kassi, Vincent Djohan, William Yavo and Hervé Menan
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, alkaloids, 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 fungidal 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, we bought them from sellers in different markets of Abidjan (Abobo, Adjame 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.
adscedens*, *Z. zanthoxyloides* Lam. and *M. micrantha* L. The plant
materials were the bark of *Pycnanthus angolensis*, the creeping
stems of *Desmodium adscedens*, 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. adscedens, 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

**MATERIALS AND METHODS**

**Plants collection**

For the identification of medicinal plants used to treat superficial

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Table 1. Parts of plants and their uses.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Family</th>
<th>Used parts</th>
<th>Form of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bridelia ferruginea</td>
<td>Euphorbiaceae</td>
<td>Roots</td>
<td>Maceration, decoction</td>
</tr>
<tr>
<td>Citrus aurantium</td>
<td>Rutaceae</td>
<td>Leafs, fruits</td>
<td>Cataplasm, maceration, decoction</td>
</tr>
<tr>
<td>Desmodium adscendens</td>
<td>Fabaceae</td>
<td>Leafs, Roots bark</td>
<td>Decoction, toothbrush</td>
</tr>
<tr>
<td>Pycnanthus angolensis</td>
<td>Myristicaceae</td>
<td>Stem bark</td>
<td>Decoction, cataplasm</td>
</tr>
<tr>
<td>Mareya micrantha</td>
<td>Euphorbiaceae</td>
<td>Leafs</td>
<td>Decoction, cataplasm</td>
</tr>
<tr>
<td>Zanthoxylum zanthoxyloides</td>
<td>Rutaceae</td>
<td>Leafs</td>
<td>Decoction, toothbrush</td>
</tr>
</tbody>
</table>

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 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 regeratednated at 40°C and was mixed with the inoculum at the proportion of 0.5 mL of inoculum in 25 mL of melted 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°C for 24 h. After this time, the silica plate G was removed from the oven and sprayed with 4 mL of thiazoly blue solution (2.5 mg/mL of thiazoly 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 anticandidosic 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.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Extract</th>
<th>Sterols / Polyterpenes</th>
<th>Polyphenols</th>
<th>Flavonoids</th>
<th>Tanins</th>
<th>Quinonics Substances</th>
<th>Alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bridelia ferruginea</td>
<td>H2O</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Citrus aurantium</td>
<td>H2O</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mareya micrantha L</td>
<td>H2O</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Desmodium adscendens</td>
<td>H2O</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pycnanthus angolensis</td>
<td>OH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Zanthoxylum zanthoxyloides</td>
<td>H2O</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+, 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.).

<table>
<thead>
<tr>
<th>Plants</th>
<th>Extract</th>
<th>Concentrations (mg/l)</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bridelia ferruginea</td>
<td>H2O</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>13 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>100</td>
<td>11 ± 2</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>13 ± 2</td>
<td></td>
</tr>
<tr>
<td>Pycnanthus angolensis</td>
<td>H2O</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>13 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>100</td>
<td>11 ± 2</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>13 ± 5</td>
<td></td>
</tr>
<tr>
<td>Zanthoxylum zanthoxyloides</td>
<td>H2O</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>13 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>100</td>
<td>11 ± 2</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>11 ± 4</td>
<td></td>
</tr>
<tr>
<td>Miconazole</td>
<td>100</td>
<td>11 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

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, anti diarrhoeal, 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 anticaner 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 antifungal activity of flavonoids (Seleem et al., 2017; Ozcelik et al., 2006; Orhan et al., 2010). Kamdem Wabo et al. tested the antifungal 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.

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