Antifungal activity of the extracts and saponins from Sapindus saponaria L.

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ABSTRACT

Extracts from the dried pericarp of Sapindus saponaria L. (Sapindaceae) fruits were investigated for their antifungal activity against clinical isolates of yeasts Candida albicans and C. non-albicans from vaginal secretions of women with Vulvovaginal Candidiasis. Four clinical isolates of C. albicans, a single clinical isolated of each of the species C. parapsilosis, C. glabrata, C. tropicalis, and the strain of C. albicans ATCC 90028 were used. The hydroalcoholic extract was bioactivity-directed against a clinical isolate of C. parapsilosis, and showed strong activity. The n-BuOH extract and one fraction showed strong activity against all isolates tested. Further column-chromatography on silica gel separation of this fraction afforded two pure triterpene acetylated saponins: 3-O-(4-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin (1) and 3-O-(3,4-di-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α- L-arabynopyranosyl-hederagenin (2). The structures of the compounds were based on spectral data (¹H and ¹³C NMR, HSQC, HMBC and MS), and on with literature. The saponins isolated showed strong activity against C. parapsilosis.

Key words: Sapindaceae, Sapindus saponaria, saponins, antifungal activity, Candida.

INTRODUCTION

Species from Sapindaceae family are known for their traditional medicinal uses as a diuretic, stimulant, expectorant, natural surfactant, sedative, vermifuge and against stomachache and dermatitis in many parts of the world. Chemical investigations of this family have led to the isolation of saponins, diterpenes and flavonoids, among other secondary metabolites. Several saponins and acyclic sesquiterpene and diterpene oligoglycosides have been isolated as main secondary metabolites of several Sapindaceae species used in traditional oriental medicine (Cavalcanti et al. 2001).

Sapindus saponaria L. (Sapindaceae), popularly known as ‘sabão-de-soldado’ and ‘saboeiro’, is a medium-sized deciduous tree occurring in the tropics, e.g., America and India, where the fruit is used as a soap and as a remedy against ulcers, scabies, joint pain, inflammations (Braga 1984, Abdel-Wahab and Selim 1985, Corrêa 1984, Albiero et al. 2002) and skin lesions caused by fungi (Murgu and Rodrigues-Filho 2006).

Abdel-Wahab and Selim (1985) detected the presence of carbohydrates, steroids and saponins in the leaves, stems, seeds and fruits of S. saponaria L. Flavo-
noids were detected only in the stems and leaves. Tannins, essential oil and anthraquinones were detected only in the stems. β-sitosterol and α- and β-amyrin were found in the seeds; and rutin, luteolin and 4’-methoxyflavone in the seeds and leaves.

The acetylated saponin 3-β-O-[α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl] hederagenin was detected by Lemos et al. (1992) who observed antimicrobial activity against Pseudomonas aeruginosa, Bacillus subtilis and Cryptococcus neoformans.

Ribeiro et al. (1995) isolated a monodesmosidic acetylated saponin and a mixture of two monodesmosidic saponins from a methanol extract of S. saponaria fruits. The compounds showed molluscicidal activity (LC100/24h of 5-10 ppm) against the snail Biomphalaria glabrata.

Extracts of leaves and fruits of Sapindus saponaria were orally administered to rats, and the parameters of gastric secretion, evaluated after pylorus ligature, suggested that the fruits have an anti-gastric ulcer potential (Albiero et al. 2002).

C. albicans is an opportunistic pathogen that can cause local and systemic infections in predisposed persons, commonly affecting immunologically compromised patients and those undergoing prolonged antibiotic treatment. Yet, the information available on plants, particularly medicinal plants, active against this yeast has, until recently, not resulted in effective formulations for either human or animal use (Duarte et al. 2005). The other species, called non-albicans, are more difficult to eradicate (Jackson et al. 2005). It has become evident that there are differences in the susceptibility pattern to the antifungal drugs among different species, which is relevant to the clinical and epidemiological point of view (Richter et al. 2005).

Vulvovaginal candidiasis (VVC) is an infection caused by abnormal growth of yeasts in the mucosa of the female genital tract. It is a frequent diagnosis in the daily practice of gynecology, with a significant increase during the last year (Ziarrusta 2001). VVC may be treated with either topical or systemic antifungal agents. Fluconazole has been considered the drug of choice because it is well tolerated, has good oral bioavailability and is efficacious against most Candida spp. yeasts; however, it is expensive.

According to existing literature, the investigation of natural products active against Candida spp. increased significantly in the last 10 years, and approximately 258 plant species, from 94 families were examined (Du et al. 2003).

In the present study, we describe the in vitro antifungal activity of extracts and subfractions from dried pericarps of the fruits of S. saponaria, as well as of the bioactivity-directed isolated saponins (1) and (2).

**MATERIALS AND METHODS**

**GENERAL EXPERIMENTAL PROCEDURES**

The NMR spectra were obtained in a VARIAN Gemini 90.05T, using deuterated solvent, TMS as an internal standard and constant temperature of 298K. IR: film NaCl plates; ESI-MS were recorded on a Micro mass Quattro LC, HRMS: CC: silica gel 60 (70-230 and 230-400 mesh); TLC: silica gel plates F254 (0.25 mm thickness).

**PLANT MATERIAL**

The dried pericarps of Sapindus saponaria L. fruits were collected in February 2004 on the campus of the Universidade Estadual de Maringá. The plant was identified by the UEM Botany Department, and a voucher specimen (No. HUM 11710) is deposited in the UEM Herbarium, Paraná, Brazil.

**ISOLATION OF THE CONSTITUENTS**

The dried pericarps of S. saponaria fruits (450.0 g) were ground and extracted with EtOH:H2O (9:1) at room temperature. The solvent was removed under vacuum at 40°C to yield an aqueous extract and a dark-brown residue. The aqueous extract from the crude hydroalcoholic extracts was lyophilized (50.1 g), and the residue from the crude extract was washed with EtOAc and subsequently with MeOH at room temperature. The evaporation in vacuum yielded 2.90 and 43.10 g for the EtOAc and MeOH residues, respectively. The MeOH extract was suspended in H2O and then extracted with n-BuOH, which by evaporation gave a solid residue (28.9 g). The aqueous and n-BuOH extracts were assayed against C. parapsilosis by broth microdilution assay, to determine the minimal inhibitory concentrations (MIC) as described below.
The active n-BuOH extract (3.0 g) was fractionated by successive column chromatographies (CC). Development of the column, with mixtures of solvents of increased polarity, from CHCl₃/MeOH/H₂O 90:9.5:0.5 v/v/v (50.0 mL) to MeOH/H₂O 85:15 v/v (50.0 mL) afforded 14 principal fractions. The resulting fractions were assayed for antifungal activity (Table I). The active fraction F (148.0 mg) was chromatographed by CC on silica gel 60 (70-230 mesh) eluted with mixtures of solvents of increased polarity, from CHCl₃/MeOH/H₂O 90:9.5:0.5 v/v/v (50.0 mL) to MeOH/H₂O 85:15 v/v (50.0 mL) in order to yield 92 fractions and to obtain the following compounds: 1 (2.0 mg), 2 (2.1 mg). Identification of these compounds showed that they were 3-O-(4-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin (1) and 3-O-(3,4-diacetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin (2). The chemical structures of compounds 1 and 2 are shown in Fig. 1. Structures were established with the use of spectroscopic methods (¹H and ¹³C NMR, HSQC, HMBC and ESI/MS) and by comparing them with literature data (Ribeiro et al. 1995, Murgu and Rodrigues-Filho 2006). The hydroalcoholic extract was bioactivity-directed against a clinical isolate of C. parapsilosis. The n-BuOH extract and a subfraction were tested against Candida albicans, C. glabrata, C. parapsilosis, C. tropicalis and C. albicans ATCC 90028. Saponins 1 and 2 were tested against a single clinical isolated of C. parapsilosis.

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Antifungal Susceptibility Testing

The MICs of all the extracts, compounds and reference antifungal compounds were determined by the broth microdilution method. Fluconazole powder (Pfizer Inc., New York, NY, USA) was included as a control, and the test was performed according to the proposed NCCLS, M27-A standard guidelines (1997) but with some modifications for adaptation to natural compounds. The yeast suspension had its turbidity adjusted by spectrophotometer reading according to a 0.5 McFarland standard at 530 nm wavelength. Testing was performed in sterile, flat-bottom 96 well microliter plates. A volume of 100 μL of this adjusted inoculum suspension was dispensed in each well, resulting in inoculum size between 0.5 and 2.5 × 100 cells.mL⁻¹ tested against each drug concentration. The plates were incubated at 35°C for 72 h. A quality-control organism was included in each test, in order to check the accuracy of the drug dilutions and the reproducibility of the results (Padua et al. 2003) The MIC was defined as the first well with a significant reduction (approximately 80%) in growth, compared to that of the positive control. The MFC was defined as the lowest concentration yielding negative subcultures. Fluconazole powder (Pfizer Inc., New York, NY, USA) was included in the test control.

RESULTS AND DISCUSSION

The MIC results of the extracts, fractions and compounds tested are shown in Table I. The in vitro results were classified according to Aligiannis et al. (2001) and Duarte et al. (2005) who proposed a classification for plant materials, as follows: when the MIC is up to 0.5 mg mL⁻¹, the antifungal activity is considered strong. If the extracts or compounds display a MIC between 0.6 mg mL⁻¹ and 1.5 mg mL⁻¹ the antifungal activity is considered moderate; above 1.6 mg mL⁻¹ the antifungal activity is considered weak.

The crude hydroalcoholic extract obtained from dried pericarps of S. saponaria fruits were assayed for antifungal properties with microdilution-technique assays, against a clinical isolate of opportunistic pathogenic yeast from vaginal secretions (C. parapsilosis) showing a strong activity with MIC of 400.0 μg mL⁻¹. On the basis of this result, the crude hydroalcoholic extract was successively extracted with EtOAc and MeOH. The

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<tr>
<th>Tested material</th>
<th>MIC (MFC) µg mL⁻¹</th>
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<tr>
<td></td>
<td>C. albicans n = 4</td>
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<tr>
<td>Hydroalcoholic extract</td>
<td>n.d.</td>
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<tr>
<td>n-BuOH extract</td>
<td>300-600 (300–600)</td>
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<tr>
<td>Aqueous extract</td>
<td>n.d.</td>
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<tr>
<td>Fraction C</td>
<td>n.d.</td>
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<td>Fraction D</td>
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<tr>
<td>Fraction E</td>
<td>n.d.</td>
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<tr>
<td>Fraction F</td>
<td>75–150 (75–150)</td>
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<tr>
<td>Subfraction F1</td>
<td>n.d.</td>
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<tr>
<td>Subfraction F2</td>
<td>n.d.</td>
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<td>Subfraction F3</td>
<td>n.d.</td>
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<td>Subfraction F4</td>
<td>n.d.</td>
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<tr>
<td>Saponin (1)</td>
<td>n.d.</td>
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<tr>
<td>Saponin (2)</td>
<td>n.d.</td>
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<td>Fluconazole</td>
<td>0.5*</td>
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n.d. = not determined; *0.5 is the average value (Individual data are: 0.25, 0.5, 1.0, 0.25 µg mL⁻¹ respectively).

MeOH extract was further suspended in H₂O and n-BuOH. The n-BuOH extract showed activity against C. albicans, C. parapsilosis, C. tropicalis, C. glabrata and C. albicans ATCC 90028. The results showed values of MIC from 300.0 to 600.0 µg mL⁻¹ against all the yeasts tested, strong activity against C. albicans and C. tropicalis, and moderate activity against C. glabrata, C. parapsilosis and C. albicans ATCC 90028. On the other hand, the aqueous extract was inactive with a MIC > 1600 µg mL⁻¹. n-BuOH extract (3.0 g) was subjected to column chromatography on silica gel to yielding some fractions. Fraction F showed significant antifungal activity, with MIC from 75.0 to 150.0 µg mL⁻¹, and its activity against C. tropicalis was greater than against the other yeasts. Using activity-guided fractionation, antifungal saponins (1) and (2) were isolated from the Fraction F by separation by column chromatography on silica gel from the n-BuOH extract, with MIC 250.0 and 70.0 µg mL⁻¹ against C. parapsilosis, respectively. The minimal fungicidal concentrations were twice the MIC for these organisms (Table I).

Our data, in agreement with an investigation of Serjania salzmanniana (Sapindaceae) for biologically active substances, led to isolation of two saponins: salzmannianoside A (3 - O[(β-D - glucopyranosyl - (1 → 4)] - [α-L-rhamnopyranosyl - (1 → 2)] - α - L-arabinopyranosyl]gypsogenin) and salzmannianoside B (3 - O[(β-D - glucopyranosyl - (1 → 4)] - [α-L-rhamnopyranosyl - (1 → 3)] - α - rhamnopyranosyl - (1 → 2) - α - L-arabinopyranosyl]hederagenin), both active against the human pathogenic fungi Cryptococcus neoformans, Candida albicans and Aspergillus fumigatus (Ekabo and Farnsworth 1996). The saponin structures and the MIC values reinforce the results of this study.

Studies on the antimicrobial properties detected in extracts of S. mukurossi and acetylated saponin of S. saponaria, showed that they were active against Candida albicans and Cryptococcus neoformans ATCC 32264,
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OOH

O OH

OOH

OH O

HR1

O

O

O

OH

OH

OH

Saponin

Saponin 1

R1 = OH

Saponin 2

R1 = OAc

Fig. 1

as well as against the bacteria Pseudomonas aeruginosa ATCC 15422, Bacillus subtilis ATCC 6633 and Staphylococcus aureus (Lemos et al. 1992, Ahmad and Beg 2001).

Phytochemical analyses of several species of the genus Sapindus showed that they are rich in triterpenoid saponins possessing oleanolic acid and hederagenin as the genin (Murgu and Rodrigues-Filho 2006).

In recent years, triterpenoid saponins, with hederagenin or oleanolic acid as aglycone, have been found to possess antifungal activity against C. glabrata, C. albicans, Trichosporon beigeli, Penicillium avelaneum UC-4376, Pyricularia oryzae, Cryptococcus neoformans, Coccidioides immitis and Saccharomyces cerevisiae, as well as against the dermatophytes Microsporum canis and Trichophyton mentagrophytes (Du et al. 2003, Lee et al. 2001).

The saponins belong to the olean-type triterpenoid saponins, with C-28, C-30 dicarboxylic groups and olefinic double bond on C-12, which also showed antifungal activity against a panel of human-pathogenic opportunistic fungi (Escalante et al. 2002).

Despite advances in antifungal therapies, many problems remain to be solved for most antifungal drugs available. Therefore, the azole drugs, especially fluconazole, are widely used to fight C. albicans infections. Not surprisingly, repeated fluconazole therapy for fungal infections in patients, such as vaginitis in women with C. albicans infections, has been associated with an increase in azole resistance, and the survey showed that 33.5% of clinical C. albicans isolates from women with vaginitis were resistant to fluconazole. In addition, several other Candida species, such as C. krusei and C. tropicalis, are inherently resistant to fluconazole. It is therefore very important to find antifungal drugs with novel chemical structures (Zhang et al. 2005).

In vitro studies have demonstrated that most C. non-albicans species from vaginal secretions are less sensitive than C. albicans to both topical and systemic antifungal drugs (Lynch et al. 1996). In the present study, the extract and subfractions of S. saponaria were more effective against C. non-albicans, (C. tropicalis and C. parapsilosis), suggesting that the dried pericarp of S. saponaria fruits is a potential candidate to supply phytotherapeutic inhibitors of C. non-albicans growth.

The findings revealed that the antifungal properties
of the extract of dried pericarp of \textit{S. saponaria} fruits provide preliminary scientific validation for the traditional medicinal use of this plant, as a potential phytotherapeutic agent in certain fungal diseases and for the control of fungi in the environment. In addition, the extracts and fractions obtained from \textit{S. saponaria} showed a fungicidal effect. This is very promising, considering that fluconazole, the medicine of choice in VVC treatment, exerts fungistatic activity. However, the extracts and active compound isolated from \textit{S. saponaria} should be further studied in animal models in order to evaluate their \textit{in vitro} efficacy and toxicity.

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\textbf{REFERENCES}


