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Anti-*Candida* and anti-*Cryptococcus* evaluation of 15 non-alkaloidal compounds from *Pterogyne nitens*

Caroline Sprengel Lima[1], Carlos Roberto Polaquini[1], Mariana Bastos dos Santos[1], Fernanda Patrícia Gullo[2], Fernanda Sangalli Leite[2], Liliane Scorzoni[2], Vanderlan da Silva Bolzani[3], Maria José Soares Mendes-Giannini[2], Ana Marisa Fusco-Almeida[2], Andréia Alves Rezende[4], Luis Octavio Regasini[1*]

[1]*Laboratory of Green and Medicinal Chemistry, Department of Chemistry and Environmental Sciences, Institute of Biosciences, Letters and Exact Sciences, São Paulo State University (UNESP), São José do Rio Preto, Sao Paulo, Brazil*

[2]*Department of Clinical Analysis, School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara, São Paulo, Brazil*

[3]*Department of Organic Chemistry, Institute of Chemistry, São Paulo State University (UNESP), Araraquara, São Paulo, Brazil*

[4]*Department of Biology and Animal Sciences, Faculty of Engineering, São Paulo State University (UNESP), Ilha Solteira, São Paulo, Brazil*

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ABSTRACT

Objective: To evaluate anti-*Candida* and anti-*Cryptococcus* activities of 15 non-alkaloidal compounds from *Pterogyne nitens* Tulasne (Leguminosae), a South American medicinal plant.

Methods: Compounds were submitted to antifungal assays, using microdilution method described by Clinical and Laboratory Standards Institute document, with minor modifications. Five species of *Candida* and two species of *Cryptococcus*, including clinical isolates were screened. Antifungal activity was expressed by minimum inhibitory concentration (MIC). Amphotericin B and fluconazole were used as standard antifungal drugs.

Results: Among tested compounds, six substances presented fungal growth inhibition (MIC < 31.2 μg/mL) [three flavone derivatives (1–3), a glycosylated flavonol derivative (5) and two phenolic acids (10 and 12)]. Sorbifolin (1), exhibited potent antifungal activity, demonstrating MIC value of 3.90 μg/mL against *Candida glabrata* ATCC 90030, *Cryptococcus gattii* 118 and fluconazole-resistant clinical isolate of *Cryptococcus neoformans* var. *grubii*. Pedalin (2) and nitensoside B (3), two glycosylated flavone derivatives, were active against *Cryptococcus neoformans* ATCC 90012 (MIC = 7.80 μg/mL).

Conclusions: Flavone derivatives from *Pterogyne nitens* can serve as prototypes for the design and development of innovative anti-*Candida* and anti-*Cryptococcus* hits.

1. Introduction

In the last decades, there has been a significant increase in the incidence and prevalence of opportunistic fungi infections, including candidiasis and cryptococcosis. This increase is related to the growing number of immunocompromised patients, including those with AIDS, cancer, transplant recipients and premature neonates [1,2]. Seven *Candida* species are classified as having major clinical relevance, namely, *Candida albicans* (*C. albicans*), *Candida tropicalis* (*C. tropicalis*), *Candida glabrata* (*C. glabrata*), *Candida parapsilosis* (*C. parapsilosis*), *Candida krusei* (*C. krusei*), *Candida stellatoidea* and *Candida kyfer* [3–6]. Candidiasis, the most common opportunistic yeast infection in the world has been in majority with *C. albicans*. This yeast is a causative agent of mucocutaneous and vulvovaginal infections, among other more invasive infections, such as septicemia, endocarditis, meningitis and peritonitis [3,4,7]. Cryptococcosis is an important globally systemic mycosis and the third most

*Corresponding author: Luis Octavio Regasini, Laboratory of Green and Medicinal Chemistry, Department of Chemistry and Environmental Sciences, Institute of Biosciences, Letters and Exact Sciences, São Paulo State University (UNESP), São José do Rio Preto, Sao Paulo, Brazil.
Tel: +55 17 3221 2362
E-mail: regasini@ibilce.unesp.br

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prevalent disease in AIDS patients [8]. The most common clinical manifestation is cryptococcal meningitis, which has been mainly caused by *Cryptococcus neoformans* (*C. neoformans*) and *Cryptococcus gattii* (*C. gattii*). However, there are reports of human infections caused by *C. albidus* and *Cryptococcus laurentii* [9].

On the other hand, the inefficacy of conventional antifungal drugs against resistant strains, as well as their severe side effects, limited spectrum of action and drug–drug interactions justify the urgent search for novel antifungal compounds [10]. In this way, natural products have long been used as prototypes for design of innovative drugs, which may be useful against infectious diseases, such as artemisinin, quinine, β-lactams, aminoglycosides, tetracyclines, echinocandins, griseofulvin, *etc*. [11]. Several metabolites of diverse structural patterns have proven to be active against fungi, as well as the screening of plant extracts is a valid strategy being exploited to discover novel antifungal agents [12,13].

Pterogyne nitens Tulasne (Leguminosae) (*P. nitens*), popularly named as "bálsamo", "cocal", "amendoim-bravo", "amendoin-zeiro" and "yvi-raró" is the sole member of the genus. It is found in non-protected South America areas, belonging to the list of species recommended for conservation genetics in Brazil. Also, *P. nitens* is admired for the beauty and odor of its flowers, leaves and fruits [14]. Ethnopharmacological studies in Guarani communities revealed cold aqueous preparations from *P. nitens* stem barks have been used for the treatment of helminthic infestations, mainly against *Ascaris lumbricoides* [15]. Chemically, *P. nitens* presented a variety of compounds, including guanidine alkaloids, flavonoids (flavones, flavonols, flavan-3-ols and catechins), phenolic acids, triterpenes and sterols [16–19]. Guanidine alkaloids from *P. nitens* have demonstrated a broad spectrum of biological activities, including cytotoxic, pro-apoptotic, antibacterial and trypanocidal activity [20–25]. Flavones and flavonols from *P. nitens* exhibited myeloperoxidase inhibitory and antioxidant activities [26–29].

In our previous study, we identified antimicrobial activity of *P. nitens* extracts and their four guanidine alkaloids against *C. albicans*, *C. krusei*, *C. parapsilosis* and *C. neoformans* [30]. Our goal with present work was to evaluate anti-*Candida* and anti-*Cryptococcus* activities of 15 non-alkaloidal compounds against five *Candida* species and two *Cryptococcus* species.

2. Materials and methods

2.1. Non-alkaloidal compounds from P. nitens

Flavonoids (flavone, flavonol and catechin derivatives) (1–8) and phenolic acids (9–13) were isolated and identified, using chemical procedures reported previously (Figure 1). Flavone derivatives, sorbifolin (1), pedalin (2) and nitensoside B (3), were isolated from leaves [26]. Flavonol derivatives, quercetin (4), isoquercitrin (5), quercetin 3-*O*-sophoroside (6) and rutin (7) were obtained from fruits and flowers [27,31]. Ourateacatechin (8) and the phenolic acids (9–13), such as caffeic acid (9), ferulic acid (10), sinapic acid (11), chlorogenic acid (12) and gallic acid (13) were isolated from flowers [18].

Triterpene acids (14) and (15) were purified from *P. nitens* leaves for the first time. Leaves of *P. nitens* were collected from Institute of Biosciences, Letters and Exact Sciences, São Paulo State University, São José do Rio Preto, Sao Paulo, Brazil (20°47′02.4″ S, 49°21′36.0″ W) in July 2014 and a voucher specimen (10291) was deposited in the Herbarium of Ilha Solteira (HISA) of Faculty of Engineering, Ilha Solteira, São Paulo, Brazil. Shade-dried leaves (600 g) were ground and extracted with hexane (1.8 L × 3, at room temperature). Dry hexane extract (10 g) was subjected to purification by successive chromatography columns over silica gel, eluted with mixtures of hexane and ethyl acetate, as well as furnishing betulinic acid (14 and 20 mg) and oleanonic acid (15 and 14 mg) (Figure 1). Structures of compound 14 and 15 were identified according to literature data, including ^{13}C nuclear magnetic resonance spectrum analysis [32].

2.2. Microorganisms

Six ATCC biological standards were used in our preliminary experiments, including *C. albicans* ATCC 90028, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019, *C. glabrata* ATCC 90030, *C. tropicalis* ATCC 750 and *Cryptococcus neoformans* var. *grubii* (*C. neoformans* var. *grubii*) ATCC 90012. Two clinical isolates of *C. neoformans* var. *grubii*, fluconazole-resistant [*C. neoformans* clinical resistant (CnR)] and fluconazole-susceptible [*C. neoformans* clinical susceptible (CnS)], were obtained from AIDS patient with recurrent cryptococcosis [33]. Fluconazole-resistant isolate of *C. gattii* (118) was obtained from psittacine birds [34]. All yeasts were obtained from the collection of Laboratory of Clinical Mycology, Department of Clinical Analyses, School of Pharmaceutical Sciences, Universidade Estadual Paulista (UNESP), Araraquara, São Paulo, Brazil.

2.3. Minimum inhibitory concentration (MIC)

Dissolution of compounds was performed with dimethylsulfoxide on 96-well plates and their concentration ranged from 250.00 to 0.48 μg/mL. Anti-*Candida* and anti-*Cryptococcus* activity experiments were carried out using reference broth microdilution method, as outlined in M27-A3 document produced by Clinical and Laboratory Standards Institute [35], with minor modifications [36]. Amphotericin B and fluconazole (FCZ) were used as standard antifungal drugs. MIC values were determined as the lowest concentration of test samples which showed complete fungal growth inhibition. Some 96-well plates were analyzed visually and spectrophotometrically. All tests were performed in triplicate and in the three independent experiments.

3. Results

MIC values for all yeasts were given in Table 1. Out of 15 non-alkaloidal compounds (1–15), six substances presented fungal growth inhibition (MIC $\leq$ 31.20 μg/mL) including three flavone derivatives (1–3), a glycosylated flavonol derivative (5) and two phenolic acids (10 and 12).

Compound 1 demonstrated potent antifungal activity against both human opportunistic fungi, with MIC values ranging from 3.90 to 31.20 μg/mL. In anti-*Candida* assays, the most potent effect of compound 1 was against *C. glabrata* (MIC = 3.90 μg/mL), followed by *C. krusei* and *C. parapsilosis* (MIC = 7.80 μg/mL). The lowest potency of compound 1 was against *C. albicans* and *C. tropicalis* (MIC = 31.20 μg/mL). In the anti-*Cryptococcus* assays, compound 1 was active against three strains of *C. neoformans* var. *grubii* (MIC values of 3.90 and 7.80 μg/mL), including fluconazole-resistant clinical isolate (CnR). For CnR strain, compound 1 exhibited a MIC value of

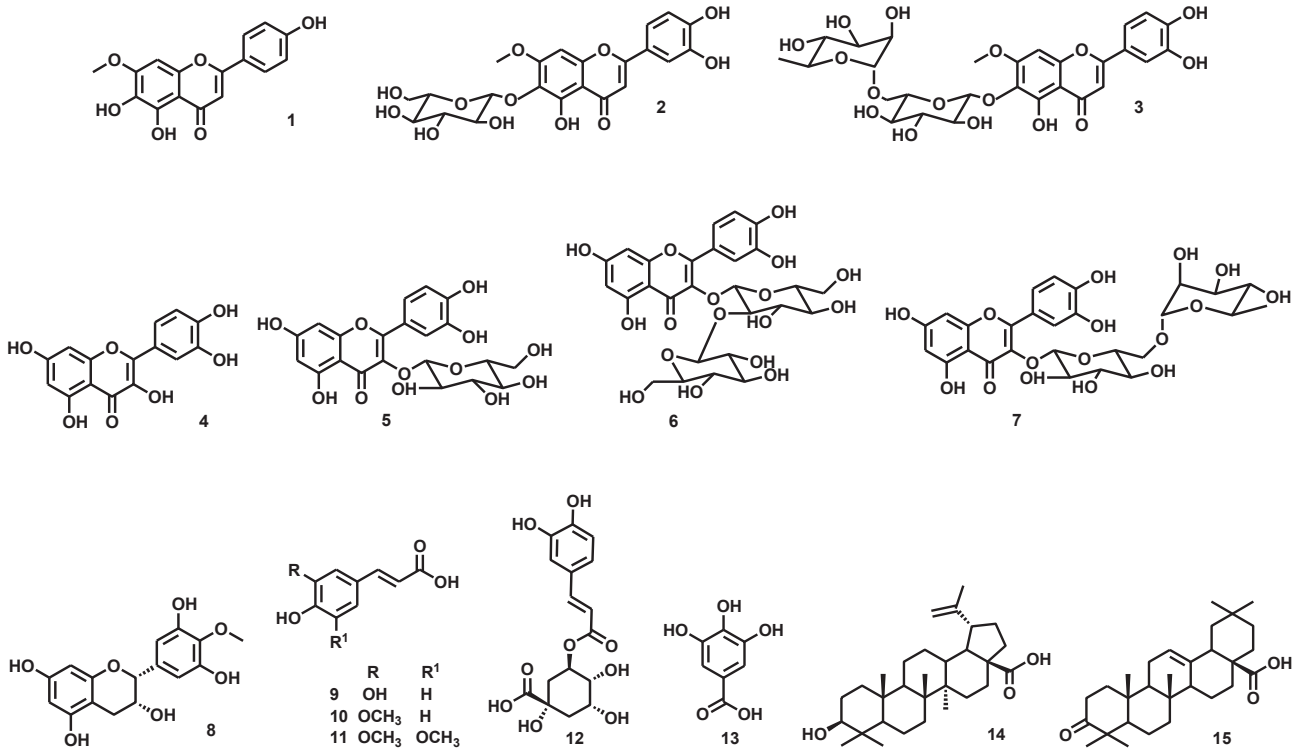

Figure 1. Structure of non-alkaloidal compounds from *P. nitens*.
1: Sorbifolin; 2: Pedalin; 3: Nitensoside B; 4: Quercetin; 5: Isoquercitrin; 6: Quercetin 3-*O*-sophoroside; 7: Rutin; 8: Ourateacatechin; 9: Caffeic acid; 10: Ferulic acid; 11: Sinapic acid; 12: Chlorogenic acid; 13: Gallic acid; 14: Betulinic acid; 15: Oleanonic acid.

3.90 μg/mL, four times less potent than amphotericin B, which has been administered as gold standard for cryptococcosis treatment [37]. Compound 1 was active against *C. gattii* (118), displaying a MIC value of 3.90 μg/mL, four times less potent than amphotericin B. For *C. neoformans* var. *grubii* ATCC 90012 and CnS strains, compound 1 (MIC = 7.80 μg/mL) was two times less potent than FCZ (MIC = 4.00 μg/mL).

Compounds 2 and 3, two glycosylated flavone derivatives, were active against *C. neoformans* var. *grubii* (ATCC 90012), with MIC values of 7.80 μg/mL, two times less potent than FCZ (MIC = 4.00 μg/mL). On the other hand, compounds 2 and 3 exhibited weak fungitoxicity against *Candida* species (MIC > 62.50 μg/mL), except compound 2 which was moderately active against *C. krusei* (MIC = 31.20 μg/mL).

Interestingly, flavonols, compound 4 and its glycosylated derivatives (5–7) were significantly less fungitoxic than flavone derivatives (1–3). Among flavonol derivatives, compound 5 demonstrated potent anti-*Cryptococcus* activity against ATCC strain, displaying a MIC value of 15.60 μg/mL, four times less potent than FCZ (MIC = 4.00 μg/mL). For this strain, compounds 4, 6 and 7 were weakly active, exhibiting MIC values of 125.00, 62.50 and 125.00 μg/mL, respectively. The comparison of MIC values for compounds 4–7 indicated number of sugar units influenced anti-*Cryptococcus* effect. Thus, order of

Table 1
MIC values of non-alkaloidal compounds (1–15) from *P. nitens*. μg/mL.

Compounds	*C. albicans* ATCC 90028	*C. krusei* ATCC 6258	*C. parapsilosis* ATCC 22019	*C. glabrata* ATCC 90030	*C. tropicalis* ATCC 750	*C. neoformans* ATCC 90012	CnS	CnR	*C. gattii* (118)
1	31.20	7.80	7.80	3.90	31.20	7.80	7.80	3.90	3.90
2	250.00	31.20	125.00	–	–	7.80	–	–	–
3	250.00	62.50	125.00	–	–	7.80	–	–	–
4	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	125.00	–	–	125.00
5	250.00	62.50	250.00	–	–	15.60	–	–	–
6	250.00	250.00	250.00	–	–	62.50	–	–	–
7	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	125.00	–	–	125.00
8	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	125.00	–	–	125.00
9	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	125.00	–	–	125.00
10	≥ 250.00	125.00	≥ 250.00	≥ 250.00	≥ 250.00	31.20	–	–	31.20
11	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	125.00	–	–	125.00
12	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	31.20	–	–	31.20
13	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	125.00	–	–	125.00
14	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	125.00	–	–	125.00
15	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	≥ 250.00	125.00	–	–	125.00
FCZ[a]	8.00	–	16.00	–	8.00	4.00	4.00	–	–
Amphotericin B[a]	–	2.00	–	0.50	–	–	–	1.00	1.00

[a]: Standard antifungal drugs.

antifungal potency was monoglycosylated (5) > diglycosylated (6 and 7) > free aglycone (4).

Among phenolic acids (9–13), compounds 10 and 12 exhibited moderate anti-*Cryptococcus* activity (MIC = 31.20 μg/mL) against *C. neoformans* (ATCC 90012) and *C. gattii* (118). Compounds 8, 14 and 15 were not active against both yeasts species (MIC ≥ 125 μg/mL).

4. Discussion

MIC values of compounds 1–3 corroborate antifungal potential of flavone derivatives, which have shown fungitoxicity against a broad spectrum of fungi species, including yeasts (*Saccharomyces cerevisiae*), halohyphomycetes (*Aspergillus*) and dermatophytes (*Trichophyton* and *Epidermophyton*) [38–40]. Nevertheless, our results to compound 1 were significantly opposite to those described by Taleb-Contini *et al.*, who reported absent growth inhibition until 500 mg/mL against *C. albicans* ATCC 1023 and *C. tropicalis* (clinical isolate from oral cavity), by using well diffusion assay [41]. This difference may be related to strain types, susceptibility tests and/or purity grade of compounds.

Review of literature data on anti-*Candida* activity of compounds 4, 10 and 13 is conflicting. A commercial sample of compound 4 presented higher anti-*C. albicans* activity (MIC = 8 μg/mL) than isolate samples from *Buddleja salviifolia* (MIC = 125 μg/mL) and *Halimodendron halodendron* (MIC = 250 μg/mL) [42–44]. Similar behavior was observed for commercial gallic acid (MIC = 8 μg/mL) in comparison to the one obtained from *Lythrum salicaria* (MIC = 2500 μg/mL), *Paeonia rockii* (MIC = 30 μg/mL) and *Pelargonium reniforme* subsp. *reniforme* (MIC = 500 μg/mL) [42,45–47]. Also, commercial sample of compound 10 (MIC = 20 μg/mL) was quite different to sample obtained from *Halimodendron halodendron* (MIC = 200 μg/mL) [44,48]. Our anti-*C. albicans* MIC value data were more similar to plant isolates than commercial samples.

Commercial samples of compounds 9 and 12 displayed MIC values of 8 and 16 μg/mL against *C. albicans* and *C. parapsilosis*, respectively [42]. In contrast, our MIC value data for compounds 9 and 12 against both yeasts were equal or superior to 250 μg/mL. Compound 7 from *P. nitens* was not able to exhibit anti-*C. albicans* activity (MIC ≥ 250 μg/mL), on the other hand, rutin commercial sample displayed the potent effect (MIC = 40 μg/mL) [48]. Martins *et al.* suggested that these differences could be probably assigned to purity grade of tested compounds [49]. Additionally, we inferred this difference may be correlated to strain types.

In summary, 15 non-alkaloidal compounds from *P. nitens* were evaluated against *Candida* and *Cryptococcus* species. Of these, compound 1 may be considered suitable template for design of innovative hits for the treatment of opportunistic yeast infections, including candidiasis and cryptococcosis.

Conflict of interest statement

We declare that we have no conflict of interest.

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