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Artigo

Chemical constituents from *Swartzia apetala* Raddi var. *glabra* and evaluation of their antifungal activity against *Candida* spp.

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RESUMO: "Constituintes químicos de *Swartzia apetala* Raddi var. *glabra* e avaliação da atividade antifúngica contra espécies de *Candida*". Do extrato hexânico da madeira de *Swartzia apetala* Raddi var. *glabra* foram isolados um estilbeno (**1**), uma flavanona (**2**), um pterocarpano (**3**), um triterpeno (**4**) e uma mistura de esteróides (**5** a **7**). O extrato bruto e as substâncias isoladas foram submetidas à avaliação do potencial antifúngico usando nove cepas padrão ATCC do gênero *Candida*. Entre as substâncias testadas apenas o triterpeno (**4**) e a mistura de esteróides (**5** a **7**) não apresentaram atividade. As estruturas das substâncias foram determinadas através da análise dos espectros de CG/EM, e RMN (1D e 2D) e comparação com dados da literatura.

Unitermos: *Swartzia apetala*, atividade antifúngica, estilbeno, flavonoides, terpenóides.

ABSTRACT: From the hexanic extract of the stem from *Swartzia apetala* Raddi var. *glabra* were isolated one stilbene (**1**), one flavanone (**2**), one pterocarpan (**3**), one triterpene (**4**) and a mixture of three steroids (**5** to **7**). The crude extract and the compounds isolated were submitted to evaluation of the antifungal activity against nine yeast standard ATCC of the *Candida* genus. Among the compounds only the triterpene (**4**) and the mixture of steroids (**5** to **7**) showed no activity. The structures of the compounds were determined by spectral data analysis of GC/MS and ^{1}H and ^{13}C NMR (1D and 2D experiments), as well as comparison with literature values.

Keywords: *Swartzia apetala*, antifungal activity, stilbene, flavonoids, terpenoids.

INTRODUCTION

The *Swartzia* genus (Fabaceae-Papilonoideae) comprehend about 130-140 species and only nine were chemically studied (Formiga et al., 1974; Braz-Filho et al., 1980; Sanchez et al., 1999; Magalhães et al., 2003; Rojas et al., 2006).

Swartzia apetala Raddi var. *glabra* is popularly known in Brazil as "arruda rajada" and has been used in civil construction and hydraulic workmanships on the basis of this large durability and impenetrability to marine worm (Rizzini and Mors, 1995), but yet without report of phytochemical investigation and evaluation of biological activity.

The yeast *Candida* is a widespread opportunistic pathogen. Mucocutaneous infections, that manifest as both oral and vaginal 'thrush' are commonly encountered and the incidence of systemic candidiasis has risen dramatically over the past decades in parallel with the increasing sophistication of medicinal technology and widespread use of aggressive therapeutic regimens for transplantation and cancer patients. With this is necessary and urgent the identification of novel bioactive compounds. *Candida* species are among the main fungi causing nosocomial infections (Rangel-Fausto et al., 1999; Aguiar et al., 2008), and other clinical human diseases special attention to HIV positive patients (Jadhav and Mishra, 2003).

This paper reports the first phytochemical study and evaluation of the biological activity from *S. apetala* Raddi var. *glabra*, allowing the isolation and characterization of the compounds (*E*)-3-hydroxy-5-methoxystilbene (3-methoxy-5-styrylphenol, **1**), (-)-5,7-dihydroxyflavanone (pinocembrin, **2**), (-)-3-hydroxy-8,9-methylenedioxypterocarpan (maackiain, **3**), 5α-lup-20(29)-en-3β-ol (lupeol **4**) and a mixture of (24-methylcholesta-5,22(*E*)-dien-3β-ol, campesterol, **5**), stigmast-5-en-3β-ol (24-ethylcholest-5-en-3β-ol, β-sitosterol, **6**) and 24-ethylcholesta-5,22(*E*)-dien-3β-ol (stigmasta-5,22(*E*)-dien-3β-ol, stigmasterol, **7**) by spectral data (GC/MS, 1D and 2D NMR) and comparison with literature values, together

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with evaluation of the antifungal activity against nine yeast standard ATCC of the *Candida* genus.

EXPERIMENTAL

General procedures

Melting points are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL Eclipse spectrometer at 400 and 100 MHz respectively, in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. Mass spectra were obtained with CGMS-QP 5050 SHIMADZU (EI, 70 eV). Optical rotation was measured with Perkin-Elmer 343 polarimeter at the sodium-D line.

Plant material

Swartzia apetala Raddi var. *glabra* was collected in Forest Reserve Vale do Rio Doce (CVRD) in November 1996, Espírito Santo, Brazil. The specimen was authenticated by the botanical identifier Domingos A. Folly by comparison with voucher of the herbarium company (nº 395).

Extraction and isolation

The dried stem of *S. apetala* (0.58 kg) was powdered and successively extracted by maceration at room temperature with hexane, CH_2Cl_2 and methanol. The hexane extract (2.3 g) was submitted to partition with EtOAc to obtain two fractions F1 and F2. The fraction soluble in EtOAc F1 was chromatographed on a silica gel column using a hexane-CH_2Cl_2-methanol gradient obtaining 38 fractions of 50 mL that were reunited of according to chromatographic profile resulting in four fractions (F1A, F1B, F1C and F1D). The fraction F1B (110 mg) was purified by column chromatography on silica gel using a hexane-CH_2Cl_2-EtOAc-methanol gradient to obtain compounds 3-methoxy-5-styrylphenol (**1**, 7.0 mg) and pinocembrin {**2**, 18.0 mg; m.p. 196-198 ºC; $[\alpha]_D$ - 57º (c 0.003, $CHCl_3$)}. The fraction F1C (355 mg) was subjected to the same procedure used to F1B, obtaining 25 fractions of 50 mL and the fractions were reunited in three fractions (F1C-1 to F1C-3). The fraction F1C-1 (21 mg) was purified by preparative TLC using hexane-CH_2Cl_2 (3:2) as eluent afforded the pterocapan maackiain {**3**, 13.0 mg; m.p. 180-185 ºC; $[\alpha]_D$ - 268º (c 0.02, $CHCl_3$)}. Fraction F1C-3 (33.5 mg) was also subjected to preparative TLC using hexane-EtOAc (4:1) as eluent and afforded triterpenoid lupeol (**4**, 13 mg; m.p. 213-215 ºC). Fraction F1D (230 mg) was chromatographed on silica gel column flash using petroleum ether-EtOAc (8:2) and obtained a mixture (35 mg).of the steroids campesterol (**5**), β-sitosterol (**6**) and stigmasterol (**7**).

Bioassay

The evaluation of the potential antifungal of the extract and the isolated compounds was evaluated by the agar diffusion method (Hadaceck & Greger, 2000). The yeasts inocula (100 µL) were spread on the surface of Sabouraud dextrose agar (Difco, USA) in 100 mm x 17 mm plates Petri dishes by using a swab (Venturi, Transystem, Copan Innovation, Italy). The colonies

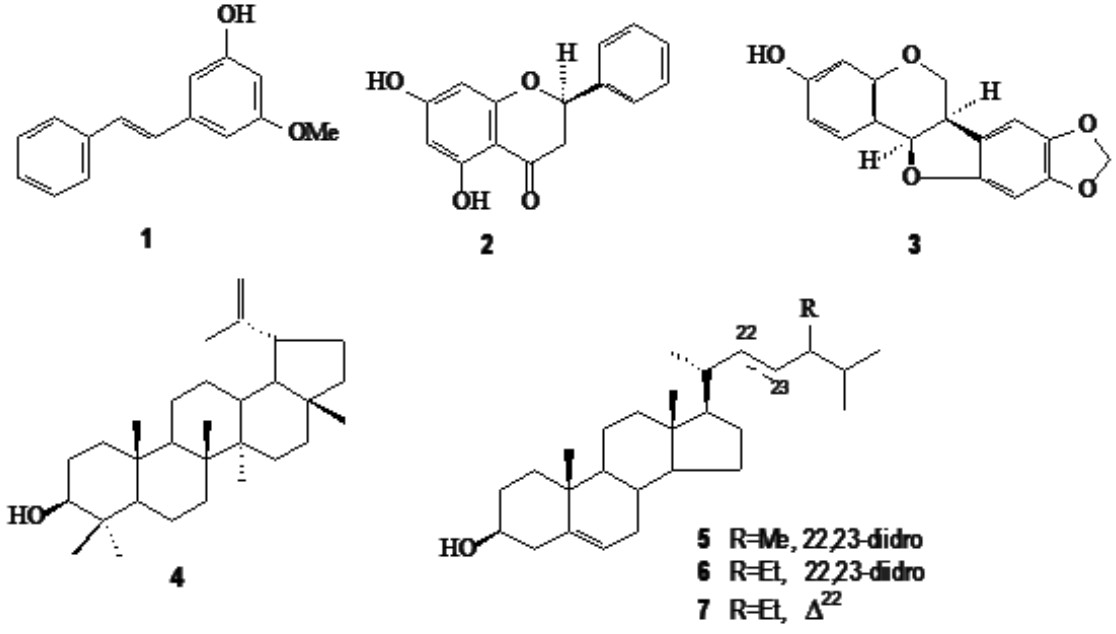

Figure 1. Compounds isolated from *Swartzia apetala* Raddi var. *glabra.*

Table 1. Extract and compounds activity of *Swartzia apetala* Raddi var. *glabra* toward *Candida* spp. measured by agar diffusion method.

Yeast strains	Extract and compounds tested/diameters of inhibition zones (mm)						
	extract	stilbene	flavanone	pterocarpan	lupeol	steroids	Positive control
C. albicans	0	15 ± 0,05	14 ± 0,05	10 ± 0,07	0	0	30 ± 0,06
C. tropicalis	13 ± 0,10	12 ± 0,18	7 ± 0,01	10 ± 0,03	0	0	25 ± 0,03
C. spp.	0	11 ± 0,07	9 ± 0,09	10 ± 0,04	0	0	28 ± 0,07
C. parapsilosis	0	16 ± 0,08	15 ± 0,06	10 ± 0,09	0	0	33 ± 1,3
C. guillermondii	18 ± 0,04	16 ± 0,09	10 ± 0,07	13 ± 0,05	0	0	40 ± 1,6
C. lusitaneae	11 ± 0,01	11 ± 0,05	11 ± 0,08	11 ± 0,04	0	0	35 ± 0,08
C. glabrata	13 ± 0,10	13 ± 0,02	5 ± 0,05	0	0	0	27 ± 0,40
C. krusei	11 ± 0,02	11 ± 0,08	9 ± 0,10	9 ± 0,01	0	0	22 ± 0,04
C. inconspicua	17 ± 0,08	14 ± 0,04	12 ± 0,11	10 ± 0,06	0	0	32 ± 0,07

yeasts were suspended in sterile saline and read by photometry (Densimat, bioMérieux, France) adjusted to nº 0.5 McFarland scale (10^6 CFU/mL). Afterwards, 5.0 mm of diameter wells were perforated and filled with 50 µL of the extract (25 mg/mL) and 50 µL of the compounds isolated (1.0 mg/mL). The plates were incubated at 37 ºC for 36 hours. All tests were carried out in triplicates. Myconazole nitrate (Vodol® batch 606401, União Química, Brazil) (50 µL; 1.0 mg/mL) was used as positive control. Solvent control (absolute ethanol) was included in each experiment as negative control. The antifungal activity was measured as the diameter (mm) of clear zone around wells containing all products tested. The yeast utilized were *Candida albicans* ATCC 36802, *C. lusitaneae* ATCC 34449, *C. guillermondii* ATCC 6260, *C. glabrata* ATCC 2001, *C. inconspicua* ATCC 16783, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 34135, *C. tropicalis* ATCC 13803 and *C. spp*. 34147.

RESULTS AND DISCUSSION

The identification of the compounds **1-7** involved comparison of the spectral data, obtained through GC/MS, 1D and 2D NMR analysis, with values described in the literature for 5-hydroxy-3-methoxystilbene **1**, (Ngo & Brown, 1998), 5,7-dihydroxyflavanone **2**, (Markham & Chari, 1976), 3-hydroxy-8,9- methylenedioxypterocarpan **3**, (Bedir et al., 1999), lupeol **4**, (Campos et al., 1991; Zanon et al., 2008) and a mixture of steroids campesterol **5**, sitosterol **6** and stigmasterol **7** (Seo et al., 1988). Two of these compounds (**1** and **2**) are being reported by first time in the *Swartzia* genus (Figure 1).

The screening of the antifungal activity *in vitro* of the hexanic extract of the stem of *S. apetala* and the compounds isolated against nine yeast of the *Candida* genus are show in the Table 1. Compared with the positive control results, the activity of the extract may be due to the synergic effect of the three active compounds together. From the results obtained with the three compounds the compounds **1** and **2** showed activity against most of yeast analyzed with inhibition zone values of ((15 ± 0.05 mm) *C. albicans*, (16 ± 0.08 mm) *C. parapsilosis* and (16 ± 0.09 mm) *C. guillermondii* for the compound **1**. Activity of the compound **2** towards *C. albicans* (14 ± 0.05 mm) and *C. parapsilosis* (15 ± 0.06 mm) were similar to compound **1**. Amongst the three compounds tested, the compound **3** was the less active showing as most significant result against *C. guillermondii* (13 ± 0.05 mm) and no inhibition against *C. glabrata*. The other compounds showed no inhibition halo.

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