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Artigo

Arrabidaea chica (HBK) Verlot: phytochemical approach, antifungal and trypanocidal activities

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RESUMO: "*Arrabidaea chica* (HBK) Verlot: abordagem fitoquímica, atividades tripanocida e antifúngica". *Arrabidaea chica* (HBK.) Verlot (Bignoniaceae) popularmente, "Pariri", é um arbusto escandente, distribuído do sul do México até a Guiana e Brasil central e é tradicionalmente indicado para tratar sintomas de inflamações e afecções da pele. Seu extrato etanólico foi quimicamente investigado e testado contra leveduras e fungos dermatófitos. A atividade tripanocida do mesmo extrato foi também avaliada. Este trabalho reporta o isolamento de três flavonóides, a inibição total do crescimento de *Trichophyton mentagrophytes* e um significante efeito tripanocida do extrato etanólico e de suas frações. Não foi detectada qualquer toxicidade aguda relevante, mesmo a uma dose de 1000 mg/kg.

Unitermos: *Arrabidaea chica*, Bignoniaceae, flavonóides, *Trichophyton mentagrophytes*, atividade antifúngica, *Trypanosoma cruzi*, efeito tripanocida.

ABSTRACT: *Arrabidaea chica* (HBK.) Verlot (Bignoniaceae) vernacular name "Pariri", is a climbing shrub, widespread from South Mexico to Guyana and central Brazil and is traditionally indicated to treat symptoms of inflammations and skin affections. Its ethanol extract was chemically investigated and tested against yeasts and dermatophytic fungi. The trypanocidal activity of the same extract was also evaluated. This work reports the isolation of three flavonoids, the total growth inhibition of *Trichophyton mentagrophytes* and a significant trypanocidal effect of the ethanol extract and its fractions. No relevant acute toxicity was detected even at a dose of 1000 mg/kg.

Keywords: *Arrabidaea chica*, Bignoniaceae, flavonoids, *Trichophyton mentagrophytes*, antifungal activity, *Trypanosoma cruzi*, trypanocidal effect.

INTRODUCTION

Arrabidaea chica (HBK) Verlot, Bignoniaceae, is a scrambling shrub which occurs in tropical America, more particularly in the Amazon basin where it is called "Pariri", "Crajiru", "Carajuru" or " Carajiru" (Correa, 1931; van den Berg, 1993).

The leaves of the plant have been traditionally used by Brazilians Indians as dye in body painting for rituals and to protect the skin against the sunlight and to repel insect. Since the beginning of this century *A. chica* has been matter of chemical investigation that aimed to determine the composition of the dye, which was commercialised at that time (Chapman et al., 1927).

Nowadays *A. chica* is widely used in the popular medicine in Northern Brazil to treat blood dysfunction (anaemia, haemorrhage) and uterine inflammation being also indicated in hepatitis, haemorrhoids and skin affections. The plant is used as an infusion of fresh or dried leaves drunk continuously during one to three days replacing the usual diary beverage, or eventually used to bath external wounds (Barbosa et al., 2001).

Until today despite the large use and indication of *A. chica* very few is known about the chemical constitution of its leaves. The analysis of the dye performed decades ago (Chapman, 1927), the isolation of a flavone (Takemura, 1995) and of three anthocyanidins (Zorn et al., 2001) are the disposable chemical data about this species. Moreover, no pharmacological studies have been reported in the literature. More recently the content in phenolics and flavonoids in the leaf were determined, ± 10.2 mg/g and 0.06 mg/g, respectively (Silva et al.,

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2007). The total flavonoids content in tinctures (30%; 50% and 70% ethanol) and in aqueous extracts (infusion and decoction were also determined (Pinto, 2004) (Table 1).

Table 1. Total flavonoid content in preparations of *A. chica*.

Sample	Conc. (g%)
Infusion	1,300
Decoct	1,842
Tincture 30%	4,866
Tincture 50%	10,489
Tincture 70%	14,969

The present article reports the antifungal and trypanocidal activities detected in the ethanol extract of *A. chica* and in its fractions. The phytochemical analysis of the ethanol extract is also reported as the isolation of three flavonoids.

MATERIAL AND METHODS

Plant material

Leaves of *Arrabidaea chica* (HBK) Verlot were collected in Belém (State of Pará, Brazil). The plant material was identified by Dr. Maria Elisabeth van den Berg at the Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, Brazil where a voucher specimen is deposited and registered under the number 150.701 (van den Berg, 1993).

Preparation of extract and fractions

Ethanol extract was prepared by maceration of 3.5 kg of fresh leaves for 5 days at room temperature. After filtration, the solvent was evaporated under reduced pressure to yield 101.0 g of a viscous red brown extract - EtE - corresponding an yeld of 2.89%. 50 g of the crude extract was then fractionated by column chromatography on silica gel (70-230 mesh ASTM, Merck™) using successively petroleum ether 100%, petroleum ether-hexane 50/50, hexane 100%, hexane-dichloromethane 50/50, dichloromethane 100%, dichloromethane-methanol 50/50 and finally methanol 100% as eluent.

Phytochemical analysis

Chemical tests to detect the main classes of secondary metabolites were carried out using classical specific colour reactions (Mattos, 1988; Steinegger & Hansel, 1992; Barbosa, 2001; Sena Filho et al., 2006; Migliato et al., 2007) and by spraying colour reagent on thin layer chromatograms (Wagner & Bladt, 1996).

The isolation of a flavonoid was achieved by direct treatment of plant material with boiling *n*-hexane and the two others using usual chromatographic techniques. They were analysed with adequate spectrometric methods in order to characterize their structures.

Antifungal activity

The antifungal activity of *A. chica* was assayed for four pathogenic fungi species using amphotericin B (Sigma) at 0.25 mg/mL as a positive control and DMSO-Tris buffer 1:9 as a negative control. Tests were done in triplicate using the agar dilution method (van den Berghe & Vlietinck, 1991; Sindambiwe et al., 1999; Longhini et al., 2007; Ostrosky et al., 2008). Clinical samples of *Candida albicans*, *Aspergillus niger*, *Trichophyton rubrum* and *T. mentagrophytes* were collected at the Clinical Laboratory (UFPA, State of Pará, Brazil) and identified by Prof. Dr. Jorge Pereira da Silva from the Faculdade de Farmácia, Universidade Federal do Pará. *T. mentagrophytes* is a frequent causal agent for several common skin infections in Brazil.

Evaluation of the trypanocidal effect

The assay was performed using the method described by Brener (1962) and Pizzolatti et al. (2008) against trypomastigotes, the blood circulating forms of the parasite. Trypomastigotes of *T. cruzi* (strain Y) were obtained from infected mice by collecting the blood at the top of the parasitemy (7^{th} day) by cardiac punction. The extract and the fractions were tested in triplicate at 4 mg/mL and 2 mg/mL respectively using a range of 2 x 10^5 parasites. Parasites were counted in a hematocytometer after incubation at 4 ^{0}C for 24 h and the counts were compared with those without drug. The efficacy (percentage of lysis) was assessed comparing the results with crystal violet (250 µg/mL) used as baseline drug.

RESULTS AND DISCUSSION

Assays performed on crude ethanol extract using specific chemical reagent gave characteristic responses, as seen on the Table 2.

Isolation of I

From 112 g fresh leaves 35 mg of (**I**) could be isolated by extraction with *n*-hexane under 6 h reflux. The substance was recovered by filtration after cooling. Yield 0,031%. m.p. 203 ^{0}C (C_6H_{14}); UV max (MeOH): 256 (lgε = 0.97), 309 (lgε = 1.16), 482 (lgε = 1.06); (MeOH-NaOH 2M): 256, 310, 481; (MeOH-$AlCl_3$): 243, 313, 473; (MeOH-$AlCl_3$/HCl): 242, 313, 473; (MeOH-NaOAc): 245, 303, 561; (MeOH-NaOAC/H_3BO_3): 308, 468; IR bands (NaCl-$CHCl_3$): 3185, 2847, 1721, 1422, 1257, 1177, 1108, 1081, 907, 815 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.90 (3H, *s*, 7-OCH_3), 4.10 (3H, *s*, 3-OCH_3), 6.55 (1H, *d, J*=1.0Hz, H-8), 6.99 (1H, *d, J*=8.0Hz, H-5), 7.02 (2H, *dd, J*=8.0 and 2.0Hz, H-3' and 5'), 7.88 (2H,

Table 2. Metabolic classes detected in the ethanol extract of *A. chica.*

Metabolic Class	Alkaloids	Anthocyanidins	Anthocyanins	Antraquinone	Cathechins	Organic acids	Reducing sugars	Steroids	Xanthones	Coumarines	Tannins	Flavanonols	Flavanone	Heart glycosides	Saponines
EtE	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND	(+)	(+)	(+)	I	ND

ND: Not determined; I: Only indication.

dd, J=7.0 and 2.0 Hz, H-2' and 6'), 8.01 (1H, *dd, J*=8.0 and 1.0 Hz, H-6); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 176 (C-4), 162 (C-7), 158 (C-D'), 156 (C-9), 139 (C-2), 135 (C-5), 133 (split C-3), 127 (C 2' and 6'), 123 (C-1'), 118 (C-10), 114 (C-3' and 5'), 102 (C-6), 98 (C-8), 60 (OCH_3 at C-3), 55 (OCH_3 at C-7); MS (70 eV) *m/z*: 300 (12%), 299 (40), 298 (m^+ 100), 297 (99), 296 (49), 295 (22), 283 (28), 269 (16), 256 (30), 255 (94), 254 (42), 253 (46), 240 (9), 237 (8), 145 (8), 116 (8), 113 (8), 98 (8), 87 (26), 86 (10), 75 (8), 74 (30), 73 (14), 70 (14), 69 (34), 68 (22), 58 (12), 57 (30), 56 (12), 55 (32).

The infrared spectrum of **I** shows characteristic bands for flavonoids (Pretsch, et al., 1990) at the following wave number (in cm^{-1}) 3185, 1422 for hydroxyl group; 2847 for methoxy group; 1721 for carbonyl group α,β-unsaturated; 1177 for γ-lactone, 1257 indicates ether group, 1108 and 1081 for methyl ether at sp^2-C. The bands at 907 and 815 correspond to 1,2,4-trisubstituted and p-disubstituted benzene ring respectively .

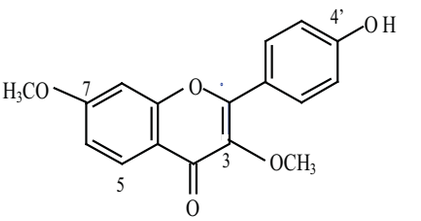
Ultraviolet spectra in MeOH indicates a flavonol (Markham et al., 1975) as base structure for **I**. Addition of NaOAc produces a bathochromic shift of 99.4 nm of the band I, indicating the presence of a hydroxyl group at C-4'. The addition of $AlCl_3$ e $AlCl_3$/HCl cause hypsochromic shift of 8,8 nm e 13 nm, and a bathochromic shift of 3,6 nm. These values did not support the existence of groups like 4-keto-5-hydroxy, 4-keto-3-hydroxy and/or *o*-dihydroxylated rings.

The MS spectrum of **I** shows base and molecular peak at m/z 298, which corresponds to $C_{17}H_{14}O_5$. The relevant signals are: $[m-1]^+$ = 297, $[m]^+$ - CH_3 = 283, $[C_{16}H_{11}O_5]^+$ - CO = 255, and 145, which corresponds to a Retro Diels Alder fragmentation of $[m-3]^+$.

The ^{13}C-NMR chemical shifts of the benzopyran system of **I** were attributed by comparison to partial structures already reported (Hattori et al., 1992, Ternai & Markham, 1976, Wagner et al., 1976) and to calculated values (Pretsch et al., 1990). The sign due to C-3 is splitted probably because this position appear to stay under influence of the anisotropic effect of the B ring (Δδ = 0.057 ppm).

The ^{1}H-NMR shows singlet at 3.90 ppm and at 4.10 ppm corresponding to two -OCH_3 groups. The coupling of H-8, H-6 and H-5 form an ABX-system. H-5 appears at 8.01 ppm, H- 6 at 6.99 ppm and H-8, at 6.55 ppm. The coupling constants are $J_{1,2}$ = 8.0 Hz and $J_{1,3}$ = 1.0 respectively. H-2'and H-6'have the same chemical and magnetic environment, therefore show the same chemical shift 7 at 7.88 ppm. The same can be observed for H-3' and H-5'. They have δ = 7,02 ppm. The coupling constant of 7.0 Hz corresponds to the *ortho* interaction of H-2' and H-3'; and of H-5' and H-6'. For the coupling between H-2' and H-6'; and H-3' and H-5', could be observed a coupling constant of 1.0 Hz.

These spectrometric data correspond to 4'-hydroxy-3,7-dimethoxyflavone (**I**).



I

This substance has no data registered in the literature and also never was described in *Arrabidaea* genus before.

Isolation of vicenin-2 and kaempferol

An aliquot of 20 g EtE was treated successively with hexane, ethyl acetate and methanol. 800 mg of the ethyl acetate fraction were submitted to column chromatography on 15 g silica gel RP18 (Merck™) using methanol/water (85:15) as eluent. Fractions 04 to 08 and 11 to 14 were analysed by TLC (SiO_2/$CHCl_3$-CH_3OH 90:10) and showed Rf 0.38 and 0.56 respectively. They were combined to furnish 7 mg vicenin-2 and 23 mg kaempferol after purification. Vicenin-2 was purified by crystallisation from methanol/chloroform (1:1) and kaempferol by preparative TLC. The substances were characterised by spectrometric methods (^{13}C-NMR, ^{1}H-NMR and UV) (Pretsch et al., 1990) by comparison with published data (Xie et al., 2003; Wagner et al., 1976).

Trypanocidal activity

Table 3. Percent of lysis of trypomastigotes forms of stem Y of *Trypanosoma cruzi* toward eight fractions of ethanol extract of *A. chica.*

Fractions	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
% of lysis	6.2	0.0	20.0	27.3	0.0	71.3	0.0	54.1

EtE was tested at a concentration of 4 mg/kg showing a significant activity on trypomastigote forms of *T. cruzi*, inducing 41% of cell lysis (Table 3). Chromatographic fractionation led to two fractions of higher activity, when tested at the concentration of 2 mg/mL: the fraction F7, eluted with CH_2Cl_2/MeOH (50:50), produced lysis in 71% of the parasite cells, while the fraction F9, eluted with MeOH 100%, produced 54% of lysis. Two others fractions, F4 and F5, both eluted by CH_2Cl_2 100% still exhibited significant, even weak, activity with 20% and ~28% of lysis, respectively.

This effect is already known for other *Arrabidaea* species and has been connected to the presence of triterpenoid acids from ursan and oleanan group (Leite et al., 1998) in the extract. The phytochemical approach also detected presence of compounds of that class in *A. chica*, however a possible role of naphtoquinones in the process of growth inhibition may not be discarded since it have been shown that the redox cycling of these substances, in *T. cruzi*, generates the highly cytotoxic hydroxyl radical. β-Lapachone, one of the first natural drugs tested has a minimum growth inhibitory concentration of 0.8 μg/mL against epimastigote forms (Oliveira et al., 1996).

CONCLUSIONS

After 14 days incubation EtE shows activity against *T. mentagrophytes* at a minimal inhibition concentration of 3.125 mg/mL. No growth inhibition could be observed for the other three species tested. The activity against the same species has been reported for other genera of Bignoniaceae and was attributed to quinones (Ali et al., 1998; Saúde-Guimarães & Faria, 2007). α-and β-lapachone, two substances from this chemical class, have already been identified in *Arrabidaea formosa* (Rocha et al., 1998). Such results allow suggesting that quinones, detected in the phytochemical approach of *Arrabidaea chica,* could also be involved in the detected antifungal activity. Furthermore, other compounds, like flavonoids, detected in EtE, and described as having antifungal activity (Prasad et al., 2004) could also be involved in the activity reported here, since flavonoids are synthesized by plants in response to microbial infection. Thus, detection of the antifungal activity against *T. mentagrophytes* supports the traditional use of this plant validating the popular indications of *A. chica* to treat skin diseases. In addition, the results of the phytochemical approach show that *A. chica* produces compounds like anthocyanidins, catechins, organic acids, reducing sugars, steroids and xanthones (Table 2). Some of these chemical compounds (Cowan, 1999) can play a supplementary role in the antifungal activity of *A. chica*.

Finally, this work contributes to validate the popular indications of *A. chica* - for skin diseases - and reports an expected trypanocidal activity

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