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Artigo

Free radical scavenging and anti-edematogenic activities of *Paullinia elegans* Cambess., Sapindaceae, leaves extracts

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RESUMO: "Atividades sequestradora de radicais livres e anti-edematogênica de extratos das folhas de *Paullinia elegans* Cambess., Sapindaceae". O extrato etanólico das folhas de Paullinia elegans Cambess., Sapindaceae, e as frações *n*-hexano, clorofórmio, acetato de etila e hidroetanólica, obtidas de seu fracionamento, foram avaliadas quanto às suas atividades anti-edematogênica e sequestradora de radicais livres. O extrato etanólico e a fração hexano produziram inibição significativa (74,4 e 76,0%, respectivamente) do edema da orelha induzido pelo óleo de cróton em ratos, em doses de 5 mg/orelha. As frações acetato de etila e hidroetanólica mostraram atividade sequestradora de radicais livres no ensaio de DPPH, com IC_{50} de 36,7 e de 30,1 µg/mL, respectivamente. O fracionamento dos extratos pelo uso de métodos cromatográficos resultou no isolamento do epifriedelanol, ácido 3-*O*-acetil oleanólico, mistura do stigmasterol 3-β-*O*-glucopiranosídeo e sitosterol 3-β-*O*-glucopiranosídeo, canferol, canferol 3,7-*O*-α-di-ramnopiranosídeo, canferol 3-*O*-α-ramnopiranosídeo e 2-O-metil-*chiro*-inositol. Os compostos foram identificados com base na comparação de seus dados espectroscópicos de RMN com os da literatura.

Unitermos: *Paullinia elegans*, atividade anti-edematogênica, atividade sequestradora de radical livre, constituintes químicos.

ABSTRACT: Ethanol extract of the leaves of *Paullinia elegans* Cambess., Sapindaceae, and its hexane, chloroform, ethyl acetate, and hydroethanol fractions were evaluated for their anti-edematogenic and free radical scavenging activities. The ethanol extract and the hexane fraction produced statistically significant inhibition (74.4 and 76.0%, respectively) of the ear edema induced by croton oil in mice, observed at doses of 5 mg/ear. The ethyl acetate and hydroethanol fractions showed significant radical scavenging effect in the DPPH assay, with IC_{50} of 36.7 and 30.1 µg/mL, respectively. Fractionation of the extracts through chromatographic methods afforded epifriedelanol, oleanolic acid 3-*O*-acetyl, a mixture of stigmasterol 3-β-*O*-glucopyranoside and sitosterol 3-β-*O*-glucopyranoside, kaempferol 3,7-*O*-α-dirhamnopyranoside, kaempeferol-3-*O*-α-rhamnopyranoside and 2-*O*-methyl-chiro-inositol. The compounds were identified on the basis of their NMR spectral data and comparison with those of literature.

Keywords: *Paullinia elegans*, anti-edematogenic activity, free radical scavenging activity, chemical constituents.

INTRODUCTION

Paullinia elegans Cambess., Sapindaceae, is found to occur in Brazil, Uruguay, Argentine and Paraguay. In Brazil, this species is known as "cipó-timbó". Chemical studies of the seeds of *Paullinia elegans* describe the isolation of *cis*-13-eicosenoic acid, *cis*-11-octadecenoic acid and 2,4-dihydroxy-3-methylenebutyronitrile (Spitzer, 1995, 1996). Species of the genus are known to contain fatty acids (Spitzer, 1996), and purin alkaloids (Weckerle et al., 2003) as major constituents.

Our preliminary studies on the pharmacological properties of *P. elegans* showed that the ethanol extract of the leaves presented significant inhibition (79.6±0.85%, at 10 µg/mL) of the growth of *Trypanosoma cruzi* epimastigotes (Truiti et al., 2005), and reduction of the volume of pleural inflammatory exudates in carrageenan induced pleurisy model in mouse, at doses of 500 $mg.kg^{-1}$ (Truiti et al., 2006).

In the present study we report the results of the

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free radical scavenging and anti-edematogenic activities evaluation of the ethanol extract and its fractions from *Paullinia elegans* leaves. The chemical composition of the fractions was investigated through chromatographic methods and the isolated compounds were characterized by NMR spectral data and comparison with those reported in literature.

MATERIAL AND METHODS

General experimental procedures

NMR spectra were recorded on a Varian Mercury Plus BB spectrometer operating at 300 MHz for ^{1}H and 75.5 for ^{13}C, using $CDCl_3$, D_2O or CD_3OD as solvents and TMS as internal standard. Columns chromatographies were performed using Sephadex LH-20 or silica gel 60 Merck (70-230 mesh ASTM). TLC was performed on precoated silica gel 60G or $60GF_{254}$ Merck.

Plant material

Paullinia elegans Cambess., Sapindaceae, leaves were collected in March 2000 in Porto Rico, Paraná, Brazil. The plant was identified by Dr. Maria Conceição de Souza and a voucher specimen (HNUP 463) has been deposited at the Herbarium of the Biology Department, State University of Maringá, Paraná, Brazil.

Animals

Male Swiss mice, weighing between 25 and 35 g were used in the experiments. The animals were kept in boxes for 72 h at room temperature, on a 12 h day-night cycle, with food and water *ad libitum*. The experimental protocols were approved by the Committee for Ethics and Animal Experimentation of the State University of Maringá.

Extract preparation and fractionation

Air-dried leaves of *P. elegans* (395 g) were exhaustively extracted by maceration with 95% ethanol at room temperature. Evaporation of the solvent afforded the ethanol extract (35 g). Part of this extract (12.6 g) was dissolved in EtOH-H_2O 1:1 and partitioned with *n*-hexane, chloroform and ethyl acetate. The solvent was evaporated to give the hexane (1.8 g), chloroform (2.8 g), ethyl acetate (3.0 g) and hydromethanol (5.0 g) fractions.

Isolation of the constituents

The n-hexane fraction (1.58 g) was purified on chromatographic column of silica gel, eluting with a mixture of *n*-hexane:ethyl acetate in increasing polarity, to afford epifriedelanol (13.5 mg) and 3-*O*-acetyl oleanolic acid (99.0 mg). The chloroform fraction (1.8 g) was submitted to a chromatographic column on silica gel, eluting with a mixture of *n*-hexane, ethyl acetate and methanol in increasing polarity, to give a mixture of stigmasterol 3-*O*-β-glucopyranoside and sitosterol 3-*O*-β-glucopyranoside (154 mg). Purification of the ethyl acetate fraction (700 mg) on a silica gel chromatographic column, eluting with a mixture of chloroform and ethanol in increasing polarity, afforded kaempferol (31.2 mg), kaempferol 3,7-*O*-α-dirhamnopyranoside (22.4 mg) and kaempeferol-3-*O*-α-rhamnopyranoside (10.5 mg). The hydroethanol fraction (504 mg) was purified on Sephadex LH-20 to give the same compounds isolated from ethyl acetate fractions together with 2-*O*-methyl-*chiro*-inositol (60 mg).The isolated compounds were identified by comparison of their NMR data with those reported in the literature (Agrawal, 1989; Mahato & Kundu, 1994; Kundu et al., 2000; Abraham et al., 2005).

Anti-inflammatory assay

Edema was induced in mice by applying 20 µL of croton oil solution (10 µg/µL) dissolved in 7:3 acetone/water, to the inner surface of both ears, according to Van Arman (1974). A solution (20 µL) of the extract and fractions (0.25 mg/µL), or of indomethacin (0.05 mg/µL) was applied to the inner surface of the left ear. The same volume of the solvent of each solution was applied to the right ear as a control. After 6 h, the animals were killed and the ears sectioned in discs of 6.0 mm of diameter each. The discs were weighed in an analytical balance for the determination of the edema inhibition percentage.

DPPH free radical scavenging assay

Free radical scavenging activities of the ethanol extract and of the n-hexane, ethyl acetate, and hydroethanol fractions were determined using 1,1-diphenyl-1-picrylhydrazyl free radical (DPPH) method. Various concentrations of the samples were added to 3 mL of daily-prepared methanol DPPH solution (0.1 mM). The mixture was shaken and left to stand at room temperature in the dark. After 30 min, absorbance was measured at 517 nm against a blank containing all reagents except the test samples. BHT was used as the positive control. Assays were carried out in triplicate.

Statistical analysis

The results for anti-edematogenic activity are presented as mean±standard error of the mean (SEM). The data were submitted to analysis of variance (ANOVA), followed by Tukey's test. $p<0.05$ was considered as the significance level.

RESULTS AND DISCUSSION

Application of croton oil to the left ear of the mice induced a very evident inflammatory response by hour 6. The weight of the ear doubled was compared to the right ear (basal, with no croton oil applied). The effects of the ethanol extract and its fractions on croton oil-induced rat ear edema are shown in Table 1 and Figure 1. The topic application of ethanol extract and hexane fraction produced a significant anti-inflammatory effect ($p<0.001$) compared to the control groups. The hexane fraction showed a percentage of 76% of inflammation inhibition, which was comparable to that of indomethacin (83% of inhibition). Esters of fatty acids, epifriedelanol and 3-*O*-acetyl oleanolic acid were isolated from the hexane fraction. The results indicate that the anti-inflammatory activity of the plant can be attributed to these compounds.

The data for free radical scavenging assay are reported in Table 2. The IC_{50} (Table 2) correspond to the concentrations for 50% of inhibition of DPPH and were calculated from the graph of I% (inhibition percentage) versus extract concentration in µg/mL. The percentage of inhibition of DPPH (I%) was calculated using the equation: I%: $(A_{blank}-A_{sample}/A_{blank}) \times 100$, where A_{blank} is the absorbance of the blank solution and A_{sample} is the absorbance of the ethanolic extract or fractions test samples.

The ethyl acetate and hydroethanol fractions exhibited the highest free radical scavenging activity with IC_{50} of 36.7 and 30.1 µg/mL, respectively. The flavonoids kaempferol and its glycoside derivatives, kaempferol 3,7-*O*-α-dirhamnopyranoside and kaempeferol-3-*O*-α-rhamnopyranoside were identified as the main components of these fractions. The hydroethanol fraction afforded also the sugar 2-*O*-methyl-*chiro*-inositol. The antioxidant effects observed for ethyl acetate and hydroethanol fractions are probably due the presence of kaempferol, since the DPPH free radical activity of this flavonoid has been reported in the literature (Seyoum et al., 2006).

Table 1. Antiinflammatory activity of *Paullinia elegans* Cambess., Sapindaceae, crude ethanol extract and its fractions on ear edema induced by croton oil.

Samples	Doses (mg/ear)	Inhibition (%)
Ethanol extract	5.0	74.4
n-Hexane fraction	5.0	76.0
Ethyl acetate fraction	5.0	11.4
Chloroform fraction	5.0	26.6
Hydroethanol fraction	5.0	35.4
Indomethacin[a]	1.0	83.3

[a] Indomethacin was used as positive control

Table 2. DPPH free-radical scavenging activity (IC_{50}) of *Paullinia elegans* Cambess., Sapindaceae, crude ethanol extract and its fractions.

Samples	IC_{50} µg/mL (95 % confidence limit)
Ethanol extract	76.9 (74.2-79.6)
n-Hexane fraction	457.1 (399.4-535.1)
Ethyl acetate fraction	36.7 (31.2-44.2)
Hydroethanol fraction	30.1 (28.5-31.5)
BHT [a]	16.9 (14.3-20.1)

[a] BHT (butylhydroxytoluene) was used as positive control.

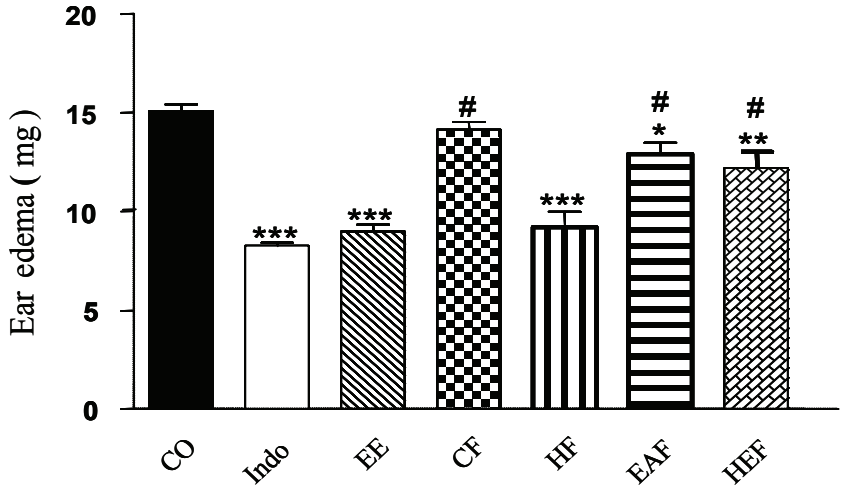

Figure 1. Effect of *Paullinia elegans* Cambess., Sapindaceae, crude ethanol extract and of the *n*-hexane (HF), chloroform (CF), ethyl acetate (EAF) and hydroethanol (HEF) fractions on ear edema induced by croton oil (CO) (200 µg) of male Swiss mice (25-35 g). Samples (5.0 mg) were administered topically. Indomethacin (Indo) (1.0 mg) was used as anti-inflammatory positive control. Each column represents the medium weight of ears±E.P.M., 6 h after croton oil application. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, compared to the control groups, # $p<0,001$, compared to Indomethacin (ANOVA, Tukey test).

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