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Anti-inflammatory Activity of the Aqueous Extract and Fractions from the Fruit of *Cayaponia cabocla* (Vell.) Mart. (Curcubitaceae)

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SUMMARY. The purpose of this work was to evaluate *in vivo* the topical anti-inflammatory activity of the aqueous extract and fractions from the fruit of *Cayaponia cabocla* (Vell.) Mart. (Curcubitaceae). Extracts and fractions from the fresh fruit of *C. cabocla* were assayed for anti-inflammatory activity by carrageenan-induced pleurisy in rats and croton oil-induced ear oedema in mice. At 250 and 500 mg Kg^{-1} the aqueous extract reduced the volume of the inflammatory pleural exudates and the number of migrated cells, and at doses of 2.5 and 5.0 mg significantly reduced the intensity of ear oedema. The mixtures of sugars were identified in the aqueous extract by analyzing the spectral data of ^{1}H and ^{13}C NMR. These data suggest that mixtures of sugars presents in the aqueous extract were responsible for reduction of the inflammatory response.

RESUMO. "Atividade Antiinflamtória do Extrato Aquoso e Frações do Fruto da *Cayaponia cabocla* (Vell.) Mart. (Cucurbitaceae)" . O objetivo deste trabalho foi avaliar a atividade anitinflamatória tópica in vivo do extrato aquoso e frações do fruto de *Cayaponia cabocla* (Vell.) Mart. (Curcubitaceae). Extratos e frações dos frutos frescos de *C. cabocla* foram avaliadas a ação antiinflamatória sobre o modelo de pleurisia induzida por carragenina e edema de orelhas de camundongos induzido por óleo de cróton. Doses de 250 e 500 mg mg Kg^{-1} do extrato aquoso reduziram o volume do exsudato pleural e o número de células migradas, e doses de 2,5 e 5,0 mg reduziram significativamente a intensidade do edema de orelha. Misturas de açúcares foram identificadas no extrato aquoso por análises espectrométricas de ressonância magnética de hidrogênio e carbono treze. Estes dados sugerem que estas misturas de açucares presentes no extrato aquoso foram responsáveis pela redução da atividade antiinflamatória.

INTRODUCTION

The herb *Cayaponia cabocla* (Vell.) Mart. (Cucurbitaceae), known as cabacinha or abóbora-do-mato, occurs widely in Brazil and is reputed in folk medicine as an anti-inflammatory, tonic, analgaesic, and a treatment for skin diseases such as herpes, acne and erysipelas [1]. No previous reports were found in the literature about pharmacological activities of *C. cabocla*. Phytochemical study of extracts of the root of *Cayaponia tayuya* revealed the presence of cucurbitacins, and these classes of compounds showed anti-inflammatory activity in several experimental models of pain and inflammation [2]. Dihydrocucurbitacin B, isolated from *C. tayuya,* reduces damage in adjuvant-induced arthritis [3]. The anti-inflammatory effect of the aqueous extract and fractions was determined by means of carrageenan-induced pleurisy, and the topical anti-inflammatory potential was determined by their ability to inhibit croton oil- induced ear oedema in mice [4]. As a reference, the non-steroidal anti-inflammatory drug indomethacin was used.

MATERIALS AND METHODS

General experiment procedures

The NMR spectra were obtained in a VARIAN GEMINI 300 (7.05T), using deuterated solvent, TMS as the internal standard and constant temperature of 298K; TLC: silica gel plates F254 (0.25 mm thickness) employing n-butanol: acetic acid: water (5:2:3) and developing with Ninhydrin and anisaldehyde/sulfuric acid reagents. Croton oil and indomethacin were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). The other reagents, of analytical grade, were Labsynth products (São Paulo, Brazil).

KEY WORDS: Anti-inflammatory activity, *Cayaponia cabocla,* Cucurbitaceae, Sugars.
PALAVRAS-CHAVES: Atividade antinflamtória, Açucares, *Cayaponia cabocla,* Cucurbitaceae.

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Plant material

Cayaponia cabocla fruits were collected in January 2004, in Maringá, state of Paraná, Brazil, identified and authenticated. A voucher specimen was deposited in the herbarium of the Department of Botany, University of Maringá (HUM 11-747). The fresh fruit pulp (1200 g) was triturated in a blender with water (1500 ml), and the extracts were filtered and lyophilized (7.0 g).

Animals used

Male Swiss mice, weighing 20-30 g, and Wistar rats, weighing 190-230 g, were used for analyses of the anti-inflammatory effect. The animals were housed in a 12 h light, 12 h dark cycle in a temperature-controlled room, with free access to water and food. The protocol of the experiments was approved and was in accordance with the guidelines of the Brazilian Committee of Animal Experimentation.

Extraction of plant material

The fresh fruit pulp (1200 g) was triturated in a blender with water (1500 ml), and the extracts were filtered. The aqueous extract was lyophilized to produce the dried extracts (7.0 g). The aqueous extract (AE) (6.0 g) was dissolved with 500 ml of water and partitioned with 500 ml of n-butanol to yield an aqueous fraction (AF) (4.0 g) and a butanolic extract (BF) (1.5 g). The BF extract (500 mg) was successively fractionated over a Lobar Merck-C-18 (250 x 10 mm), eluting with MeOH/water (1:1 v/v) to yield the largest fraction (MF) (7.6 mg).

Croton oil induced ear oedema in mouse

The oedema was induced by application of 20 µl of croton oil (200 µg) diluted in a solution of acetone/water (7:3), to the inner surface of both ears, according to Van Arman [5]. 20 µL of AE (2.5 and 5.0 mg/ear), AF (5.0 mg/ear), BF (5.0 mg/ear) and MF (2.5 mg/ear) or 20 µL of indomethacin µl mg/ear) diluted in acetone/water (7:3) and was applied to the inner surface of the left ear, and the same volume of solvent was applied to the right ear as a control. After 6 h the animals were killed, and the ears were sectioned in discs 6.0 mm in diameter and weighed (mg) in an analytical balance. The swelling induced by the phlogogen was assessed as the difference in weight (mg) between right and left. The percentage of inhibition of oedema was determined.

Carrageenan induced pleurisy in rats

Pleurisy was induced by injection of 0.25 ml of a carrageenan suspension (200 µg) into the intrapleural cavity, in the region of the right mediastinum between the 3rd and 4th ribs, according to the technique described by Vinegar *et al.* [6] The carrageenan was diluted in saline buffered with phosphate (PBS, pH = 7.4). At hour 4 after induction of the pleurisy, the animals were anaesthetised and killed, to collect the intrapleural inflammatory exudate. The material, collected by aspiration, was transferred to conical centrifuge tubes. The total volume of the exudate was measured, and a 50 µl aliquot was used to determine the number of leucocytes in a Neubauer chamber. The AE (250 and 500 mg Kg^{-1}) in a saline 0.9% vehicle or indomethacin (5 mg kg^{-1}) in a TRIS solution vehicle was administered orally by gavage, in different groups of rats, which had fasted for 15 h, 30 min prior to the induction of pleurisy.

Acute toxicity test

Male mice fasted overnight were administered intragastrically with the aqueous extract of *C. cabocla* fresh fruit pulp. Doses were increased progressively for determination of the lethal dose (LD50). The mice were observed for 7 days following treatment; food and water were provided throughout the experiment. The number of mice that died within the period of study was noted for each group, and subsequently the LD_{50} value was calculated according to the method of Miller & Tainter [7].

Statistical analysis

The results are presented as mean ± standard error of the mean (S.E.M.). 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

Acute oral toxicity of the AE extract from *Cayaponia cabocla* fresh fruit pulp was low in mice. No deaths were observed with doses up to 5000 mg/kg. The 1H and ^{13}C NMR spectrum of extracts AE, AF, BF and MF showed a signal of a complex mixture of sugars, and showed no signal of cucurbitacin. Intrapleural injection of carrageenan in of animals treated orally with saline induced an acute inflammatory response (increase in the volume of the pleural exudates and the number of leucocytes migrated), compared to the base parameters (animals which received an injection of saline in the cavity, Table 1).

Treatment of the animals with the AE extract,

Groups	Exudate volume (mL)	Leukocyte count (cells/mm^3) X10^3
Basal	0.10 ± 0.0	6700 ± 450
Control ±Cg)	0.69 ± 0.07 [a]	56750 ± 6565 [a]
Cg+AE $_{250\ mg}$	0.46 ± 0.04 [a,b]	47820 ± 2442 [a,b]
Cg+AE $_{250\ mg}$	0.43 ± 0.02 [a,b]	41500 ± 1880 [a,b]
Indomethacin	0.53 ± 0.02 [a,b]	61500 ± 6000 [a]

Table 1. Exudate volume and leukocyte number 4 after carrageenan injection (200 µg into the pleural cavity) in treated (AE or indomethacin) and non-treated rats AE: aqueous extract (250 and 500 mg/kg body wt.), Cg: carrageenan (200 µg), Ind: indomethacin (5 mg/kg body wt.). Values are mean ± SEM of 07 animals in each group. [a] p<0.001 compared to basal; [b] p<0.05 compared to control (Cg) values (ANOVA, Tukey test).

at 250 and 500 mg Kg^{-1} reduced the volume of the exudates and the number of migrated cells. Treatment of the animals with indomethacin caused a reduction in the volume of the exudates, however, it did not alter the number of migrated cells. Application of croton oil to the left ear of mice induced a very evident inflammatory response by hour 6. The AE extract at doses of 2.5 and 5.0 mg significantly reduced the intensity of edema (Fig. 1).

The percentage inhibition was 61% and 52%, respectively (Table 2). Application of the AF and BF fractions at a dose of 5.0 mg also significantly reduced edema of the ear; this effect was similar for two fractions tested (Fig. 1). Treatment with indomethacin, the positive control, caused accentuated inhibition of the inflammatory response in the ear, which differed significantly from the other treatments. The MF fraction at 2.5 mg/ear showed low activity. Several recent reports on the topical anti-inflammatory activity of plant extracts have ascribed them to cucurbitacin. Fractionation of AE extracts reduced the anti-inflammatory activity.

CONCLUSION

These data suggest that mixtures of sugars presents in the aqueous extract were responsible for reduction of the inflammatory response. The results of the study confirm the anti-inflammatory activity of plant extracts of *C. cabocla*. However, further detailed studies are required.

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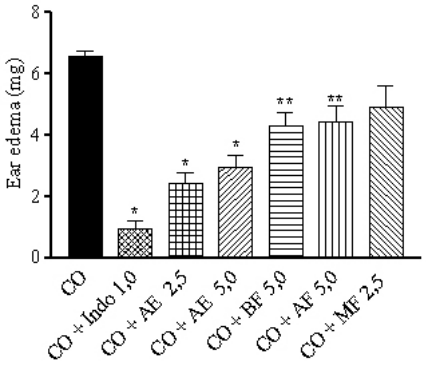

Figure 1. Effect of the extract on oedema of the ear induced by croton oil (CO) in male Swiss mice (25-35 g). The animals (n= 8 for each group) were treated topically with the AE extract and AF, BF and MF fractions, at the indicated concentrations, immediately after application of croton oil (200 µg) to the left ear. Indomethacin (Indo), 1 mg of which was administered topically, was used as the reference anti-inflammatory (positive control). Each column represents the mean weight of the ears ± SEM, 6 h after application of the croton oil. * $P < 0.01$, ** $P < 0.05$, compared to the control group (ANOVA, Tukey's test).

Groups CO	Dose (mg/ear)	Inhibition (%)
CO + Indo	1.0	84.6
CO + AE	2.5	61.0
CO + AE	5.0	52.1
CO + BF	5.0	30.9
CO + AF	5.0	28.9
CO + MF	2.5	21.9

Table 2. Percentage (%) of inhibition of ear oedema induced by croton oil (200 µg)CO: croton oil, AE: aqueous extract, BF: butanolic extract, AF: aqueous fraction, MF: majority fraction.

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