

Anti-inflammatory and antinociceptive activities of extract, fractions and populnoic acid from bark wood of *Austroplenckia populnea*

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Abstract

Austroplenckia populnea (Reiss) Lund is a Brazilian plant from “cerrado”, which belongs to Celastraceae family, popularity know as “marmelinho-do campo, mangabeira-brava, mangabarana, vime and maria-mole”. This plant is used in folk medicine to treat dysenteries and inflammatory disorders, such as rheumatism. *Austroplenckia populnea* bark hydroalcoholic crude extract, and its hexane, chloroform and ethyl acetate fractions, obtained by partition, as well as the isolated populnoic acid were investigated for their anti-inflammatory (carrageenan, dextran and histamine-induced rat paw oedema, histamine-induced increase in vascular permeability, and granulomatous tissue induction) and analgesic activities (writhing and hot plate tests). The ED₅₀ (oral) of the crude extract for the inhibition of carrageenan-induced rat paw oedema assay was determined to be 200 mg/kg, which was also used in the assays with the extract and its fractions in all other experiments. Populnoic acid was administered in the dose of 50 mg/kg. Crude extract, hexane and chloroform fractions (200 mg/kg), and indomethacin (10 mg/kg) inhibited significantly ($p < 0.05$) the formation of the carrageenan-induced rat paw oedema, measured in third hour of experiment (peak of oedema formation) by 43.2%, 37.3%, 31.1% and 59.3%, respectively. There was a significant reduction ($p < 0.05$) in dextran-induced rat paw oedema in all groups, while in the assay using histamine as the oedematogenic agent, only the groups treated with populnoic acid (50 mg/kg) and cyproheptadine (10 mg/kg) displayed significant reduction ($p < 0.05$). The populnoic acid and cyproheptadine reduced the peak of oedema formation (1st hour) by 41.3% and 34.7%, respectively. Only for the groups treated with populnoic acid (50 mg/kg) and cyproheptadine (10 mg/kg) it was observed a significant ($p < 0.05$) reduction in histamine-induced increase in vascular permeability (44.8% and 80.3%, respectively). Granulomatous tissue formation was significantly inhibited ($p < 0.05$) by both hexane fraction (46.0%) and dexamethasone (66.2%). In the analgesic assays, the crude extract and its hexane and chloroform fractions, as well as indomethacin diminished significantly the number of writhings ($p < 0.05$) by 69.6%, 47.2%, 44.8% and 62.8%, respectively. On the other hand, none assayed sample displayed significant result in the hot plate test. Based on the obtained results it is suggested that extracts of *Austroplenckia populnea* bark and populnoic acid display anti-inflammatory activity, supporting its folkloric use to treat inflammatory disorders. © 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: *Austroplenckia populnea*; Populnoic acid; Anti-inflammatory; Antrheumatic; Celastraceae

1. Introduction

Since ancient times of civilization, people have been relying on plants as either prophylactic or therapeutically arsenal to restore and maintain healthy, and plants are well know as an important source of many biologically active compounds. Recently, Rates (2001) reported that there has been a growing interest in plants as a significant source of new pharmaceuticals.

Austroplenckia populnea (Reiss) Lundell is a plant from Brazilian cerrado belonging to Celastraceae family. This family includes several plant species, which have been widely used in folk medicine for their antiulcerogenic, analgesic, male antifertility and anti-inflammatory, among other activities. *Austroplenckia populnea* is commonly known as “marmelinho-do campo, mangabeira-brava, mangabarana and vime”, and it is used in Brazilian folk medicine to treat dysenteries and especially inflammatory disorders, such as rheumatism (Côrrea, 1984; Gonzales et al., 1987; Vieira-Filho et al., 2003). Seito et al. (2002) reported the antiulcerogenic and analgesic effects in mice of the leaves hexane and methanol extracts, and Mazaro et

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al. (2000) reported the decrease in sperm number, followed by the treatment of rats with hexane extract. Studies of the chemical composition of the leaves preparations revealed the presence of sesquiterpenes and pentacyclic triterpenes (Vieira-Filho et al., 2001). In general, pentacyclic triterpenes have been reported to display anti-inflammatory, anti-ulcer, antinociceptive and antitumoral properties (Navarrete et al., 2002; Fernandes et al., 2003). Considering that it is important to fully investigate both anti-inflammatory and analgesic activities of this plant, the aim of the present study was to evaluate the anti-inflammatory and analgesic activities of the bark crude hydroalcoholic extract, its fractions and populnoic acid, a major compound isolated from the hexane extract.

2. Materials and methods

2.1. Plant material and extracts preparation

The plant material of *Austroplenckia populnea* was collected in the “cerrado” area of Botucatu, São Paulo State, Brazil. The plant material was identified by the staff of the Bioscience Institute (IBB) of the State University of São Paulo, UNESP, in which a voucher specimen (no. 20415) is deposited at the BOTU herbarium.

The wood bark was air dried at 40 °C, and the dried material (3.25 kg) was then powdered using a knife mill and exhaustively extracted by maceration with aqueous ethanol (96%), which was concentrated under vacuum, yielding 406 g of crude extract. The crude hydroalcoholic (EtOH) extract was then partitioned between hexane, CHCl₃ and ethyl acetate (EtOAc), yielding 19.0 g, 9.5 g and 98.2 g, respectively.

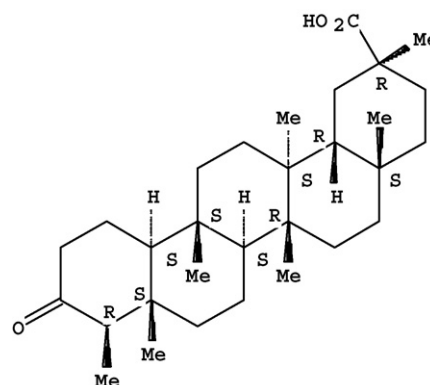
2.2. Gas chromatography analysis of the hexane extract

Chromatographic analysis was carried out using a gas chromatography (Hewlett-Packard 5890) equipped with a split/splitless injector inlet and a flame ionization detector (FID). The output was plotted and integrated to give the chromatographic data. A HP-50 capillary column (30 m in length × 0.25 mm internal diameter × 0.25 μm of film thickness) was used for all analyses. Hydrogen at a linear gas velocity of 45 cm/s was employed as carrier gas. Oven temperature program was as follows: 50–250 °C, 20.0 °C/min; 250 °C/4 min; 250–280 °C, 15 °C/min; 280 °C/18 min; 280–290 °C, 10 °C/min. The temperatures of the injection port and the detector were set at 260 °C and 330 °C, respectively. Nitrogen was used as detector make up gas at a flow rate of 30 mL/min. The flow rates for hydrogen and for synthetic air for the flame ionization detector were 30 mL/min and 350 mL/min, respectively. The injector was operated in the split mode (1/50).

The triterpenes and steroids were identified by comparison with the authentic chromatographic standards available at the compounds library of the Organic Chemistry Laboratory of the “Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP”, using both the retention times and the co-injection of the standards with the unknown samples, as parameters to identify the compounds.

2.3. Populnoic acid isolation

The hexane extract (17.0 g) was submitted to repeated column chromatography over 500 g of Si gel (9 cm × 70 cm). The elution with hexane and ethyl acetate in increasing proportions furnished 502 chromatographic fractions of 200 mL each. Thin layer chromatography (TLC) analysis of the fractions 390–407 (hexane/EtOAc 4:1) allowed to assemble them into one fraction, which was constituted of one amorphous compound, yielding 1.75 g (10.3%). Its purity was determined by TLC analysis using different solvent systems and by ¹³C NMR, which was estimated to be higher than 95%. Analysis of both RMN ¹H and ¹³C, in comparison with the data published by Itokawa et al. (1991), allowed to propose the structure of populnoic acid for the isolated compound.



2.4. Animals

Male Wistar rats and male Swiss mice weighing 200–250 g and 40–45 g, respectively, were provided by the Central Animal House of the University of Alfenas (UNIFENAS). The animals were housed in groups of five in standard cages at room temperature (25 ± 3 °C) in 12 h dark/12 h light control, with both food and water *ad libitum*. Twelve hours before the experiments they were transferred to the laboratory and were maintained only with water *ad libitum*. Animals used in the present study were housed and cared in accordance with the protocols of the University of Alfenas. Also, the experiments were authorized by the Ethical Committee for Animal Care of the University of the West of Santa Catarina, Brazil (Protocol number 045/2005), in accordance with the Federal Government legislation on animal care.

2.5. Anti-inflammatory activity

2.5.1. Determination of ED₅₀ and carrageenan-induced rat paw oedema

The ED₅₀ was determined based on the carrageenan-induced oedema. Groups of rats (n=6) were treated orally with *Austroplenckia populnea* crude extract (50 mg/kg, 100 mg/kg, 200 mg/kg, 300 mg/kg, 500 mg/kg, 1000 mg/kg and 1500 mg/kg) 30 min after the injection of the stimulus (carrageenan 1000 μg/paw) into the right hind paw planter surface. Sterile saline solution (0.9%, 0.1 mL) was injected into the left paw as the control reference for the tested paw. The foot volumes

of the animals were determined by plethymographic method described by Ferreira (1979). The inhibition of inflammation was calculated by measuring the volume difference between the right and left paws in comparison with the control group. ED₅₀ was determined from the curve drawn for the percentage of edema inhibition as a function of the dose (Carvalho et al., 1999).

The same procedure was used to evaluate the effectiveness of the extracts and populnoic acid, in the inhibition of the inflammatory process, by comparing the obtained results with both negative and positive (indomethacin) control groups. The oral administrations of the assayed samples were undertaken 30 min before the injection of carrageenan, as follow: 200 mg/kg for the crude extract and its fractions, which corresponded to the ED₅₀; 50 mg/kg of populnoic acid; 10 mg/kg of indomethacin and vehicle (0.5 mL of 3% Tween 80 solution). The foot volume measurements were taken before the injections and 3 h after the injection of the inflammatory stimulus into the right hind paw plantar surface.

2.5.2. Dextran and histamine-induced rat paw oedema

The animals were treated in a manner similar to that of carrageenan-induced paw oedema protocol, differing only in the administration of the inflammatory stimulus, which was induced by sub-plantar injection of dextran (50 µg/paw) and histamine (50 µg/paw), respectively. The oedema was measured as mentioned earlier (Winter et al., 1962) at hourly intervals for 3 h. Positive control group was treated with cyproheptadine (10 mg/kg, p.o.) in both tests.

2.5.3. Histamine-induced increase in vascular permeability

Groups of rats ($n=6$) were orally treated with extract/fractions (200 mg/kg, ED₅₀), populnoic acid (50 mg/kg), cyproheptadine (10 mg/kg) and vehicle (0.5 mL of 3% Tween 80 solution). After 30 min, the rats received an intravenous injection of Evan's blue solution (25 mg/kg). Ten minutes later, 50 µg of histamine was injected into five different points of dorsal region of each animal. Thirty minutes after histamine injection, the animals were sacrificed, and five samples of skin (1.5 cm of diameter) were cut off in areas where histamine was injected. Immediately after, the skin samples were poured into 3 mL of phormamide (98%) at 37 °C and kept for 24 h. After that, this material was filtered and centrifuged at $1000 \times g$ for 10 min and the concentration of Evan's Blue was calculated according to the absorbance measured at 620 nm using a spectrophotometer. The dye extravasation was quantified from a standard curve and expressed in µg (Mustard et al., 1965).

2.5.4. Granulomatous tissue induction

For this assay, which was described by Meier et al. (1950), seven groups of rats ($n=6$) were used. Pellets weighing approximately 40 mg each were made with 5 mm of dental cotton tampons. The pellets were sterilized and impregnated with 0.4 mL of ampicillin water solution at the moment of implantation. Animals were anaesthetized, and four pellets were subcutaneously introduced through an abdominal skin incision. Each group was treated daily, for six consecutive days p.o., with extract and its fractions (200 mg/kg, ED₅₀), populnoic acid (50 mg/kg), dex-

amethasone (0.2 mg/kg) and vehicle (0.5 mL of 3% Tween 80 solution). On the seventh day, the animals were sacrificed, the pellets were dissected out and the granulomas were dried at 60 °C overnight to determine the dried weight. The difference between the initial and final weights was considered as the weight of the granulomatous tissue produced.

2.6. Analgesic activity

2.6.1. Writhing test

The writhing test was carried out as described by Koster et al. (1959). Groups of mice ($n=8$) were treated with extract and its fractions (200 mg/kg), populnoic acid (50 mg/kg), indomethacin (10 mg/kg) and vehicle (0.5 mL of 3% Tween 80 solution), p.o. The writhings were induced by an intraperitoneal injection of a 0.6% acetic acid solution (0.25 mL/animal) 30 min after the treatment. The number of writhings was counted starting at 5 min after injection of the stimulus during 20 min. Data represent the average of the total writhings observed.

2.6.2. Hot plate test

The hot plate test was performed following the method of Jacob and Ramabadran (1978), with few modifications. Different groups of animals ($n=10$) received extract and fractions (200 mg/kg, p.o.), populnoic acid (50 mg/kg, p.o.), 0.9% saline solution (0.5 mL, p.o.) and morphine (4.0 mg/kg, i.p.). Then, mice were placed on the equipment, which was kept at 55 ± 1 °C, and the reaction time was noted by observing either the licking of the hind paws or the rotation movements at 10 min, 20 min, 30 min, 40 min, 50 min and 60 min after samples administration. Mice were selected based on the reaction time within 15 s, for which it was not observed a large variation by testing on four separated occasions.

2.7. Statistical analysis

Data are reported as mean $\pm$ S.E.M. and were analyzed statistically by analysis of variance (ANOVA) followed by Dunnett's test. Results with $p < 0.05$ were considered significant.

3. Results

3.1. Gas chromatography analysis of the hexane extract

The gas chromatography analysis of the hexane extract resulted in the identification of steroids (campesterol, stigmasterol, β -sitosterol) and triterpenes (epitaraxerol, β -amirine, lupenone, lupeol, lupeol acetate, β -friedalanol and friedelin), as displayed in Fig. 1. Populnoic acid did not elute in the used CG parameters. Both β -sitosterol and populnoic acid were major compounds present in the hexane extract.

3.2. Effectiveness median dose (ED₅₀)

The treatment with increasing crude extract doses (50 mg/kg, 100 mg/kg, 200 mg/kg, 300 mg/kg, 500 mg/kg, 1000 mg/kg and

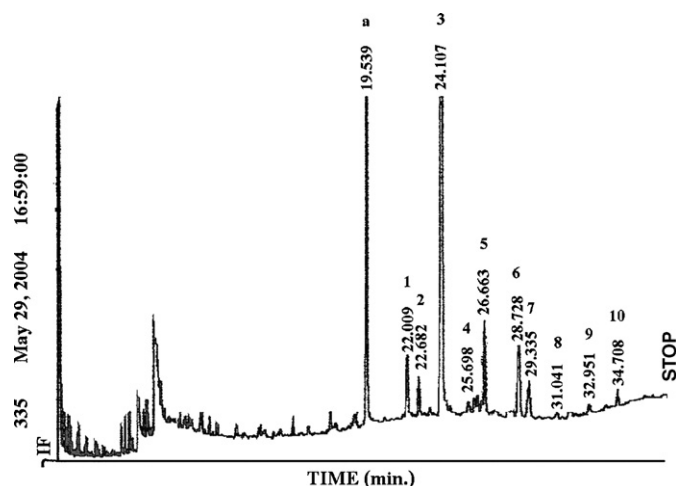


Fig. 1. CG profile of *Austroplenckia populnea* hexane extract. a: standard reference (cholesterol); 1: campesterol; 2: stigmasterol; 3: β -sitosterol; 4: epitaraxerol; 5: β -amirine; 6: lupenone; 7: lupeol; 8: lupeol acetate; 9: β -friedalanol; 10: friedelin.

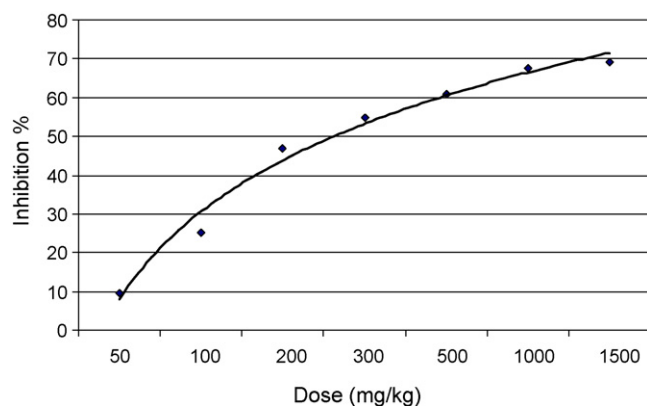


Fig. 2. Determination of the effective dose 50 (ED_{50}). Each point represents the average of six animals, expressed as inhibition percentile.

1500 mg/kg) p.o., 30 min before the administration of the inflammatory stimulus (carrageenan), produced a reduction of the oedema in a dose-dependent manner [correlation coefficient $r=0.9839$, $y=32.63 \ln(X)+7.9752$], and the ED_{50} was determined as 200 mg/kg (Fig. 2).

Table 1

Effect of the administration of crude extract, hexane, chloroform and ethyl acetate fractions, populnoic acid and cyproheptadine on dextran-induced oedema rat paw

Groups	Dose (mg/kg)	Paw volume increase			Inhibition (%)		
		1 h	2 h	3 h	1 h	2 h	3 h
Control		3.22 $\pm$ 0.26	4.01 $\pm$ 0.41	2.78 $\pm$ 0.17			
Crude extract	200	2.14 $\pm$ 0.28*	2.05 $\pm$ 0.29*	1.95 $\pm$ 0.21*	33.5	48.7*	29.8*
Hexane fraction	200	2.45 $\pm$ 0.27	1.93 $\pm$ 0.24*	1.87 $\pm$ 0.13*	23.9	51.7*	32.7*
Chloroform fraction	200	2.54 $\pm$ 0.19	2.15 $\pm$ 0.18*	1.26 $\pm$ 0.12*	21.1	46.2*	54.6*
Ethyl acetate fraction	200	1.93 $\pm$ 0.45*	1.89 $\pm$ 0.30*	1.56 $\pm$ 0.26*	40.0	52.7*	43.9*
Populnoic acid	50	2.01 $\pm$ 0.16*	2.32 $\pm$ 0.26*	1.99 $\pm$ 0.22*	37.6	42.0*	28.4*
Cyproheptadine	10	1.68 $\pm$ 0.08*	1.72 $\pm$ 0.17*	1.84 $\pm$ 0.06*	47.8	57.1*	33.8*

The results are expressed as mean $\pm$ S.E.M. of $n=6$.

* $p<0.05$, ANOVA followed by Dunnett's test.

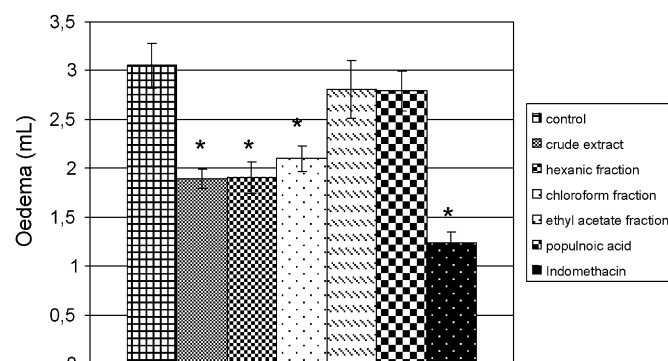


Fig. 3. Effect of the administration of crude extract, hexane, chloroform and ethyl acetate fractions (200 mg/kg), populnoic acid (50 mg/kg) and indomethacin (10 mg/kg) on carrageenan-induced rat paw oedema. The paw oedema volume was taken at the 3rd hour after carrageenan injection. Each column represents the mean $\pm$ S.E.M. of $n=6$. * $p<0.05$, ANOVA followed by Dunnett's test.

3.3. Carrageenan, dextran and histamine-induced rat paw oedema

The treatment with crude extract, hexane and chloroform fractions (200 mg/kg), as well as indomethacin (10 mg/kg) inhibited significantly ($p<0.05$) the carrageenan-induced rat paw oedema formation, which was measured at the third hour of the experiment (peak of oedema formation) by 43.2%, 37.3%, 31.1% and 59.3%, respectively (Fig. 3). The results were statistically significant in comparison to the control.

There was a significant reduction ($p<0.05$) in dextran-induced rat paw oedema in all groups, at 1st, 2nd and 3rd hours of the experiment (Table 1). Crude extract, hexane, chloroform and ethyl acetate fractions (200 mg/kg), as well as populnoic acid (50 mg/kg) and cyproheptadine (10 mg/kg) reduced oedema formation, measured at the 2nd hour (peak of oedema formation) in 48.7%, 51.7%, 46.2%, 52.7%, 42.0% and 57.1%, respectively. The results were statistically significant in comparison to the control.

There was a significant reduction ($p<0.05$) in histamine induced oedema only for the groups treated with populnoic acid (50 mg/kg) and cyproheptadine (10 mg/kg) (Table 2), which were 41.3% and 34.7%, respectively, at the 1st hour.

Table 2
Effect of the administration of crude extract, hexane, chloroform and ethyl acetate fractions, populnoic acid and cyproheptadine on histamine-induced rat paw oedema

Groups	Dose (mg/kg)	Paw volume increase			Inhibition (%)		
		1 h	2 h	3 h	1 h	2 h	3 h
Control		1.50 ± 0.18	1.25 ± 0.16	0.84 ± 0.04			
Crude extract	200	1.33 ± 0.14	1.01 ± 0.11	0.89 ± 0.09	11.3	19.2	−5.9
Hexane fraction	200	1.37 ± 0.09	1.26 ± 0.09	1.02 ± 0.13	8.7	−0.8	−21.4
Chloroform fraction	200	1.17 ± 0.25	1.18 ± 0.11	0.74 ± 0.06	22.0	5.6	11.9
Ethyl acetate fraction	200	1.23 ± 0.15	1.02 ± 0.03	0.85 ± 0.07	18.0	18.4	−1.2
Populnoic acid	50	0.88 ± 0.13*	0.88 ± 0.06*	0.76 ± 0.10	41.3	29.6	9.5
Cyproheptadine	10	0.98 ± 0.06*	0.78 ± 0.14*	0.61 ± 0.11	34.7	37.6	27.3

The results are expressed as mean ± S.E.M. of $n=6$.

* $p < 0.05$, ANOVA followed by Dunnett's test.

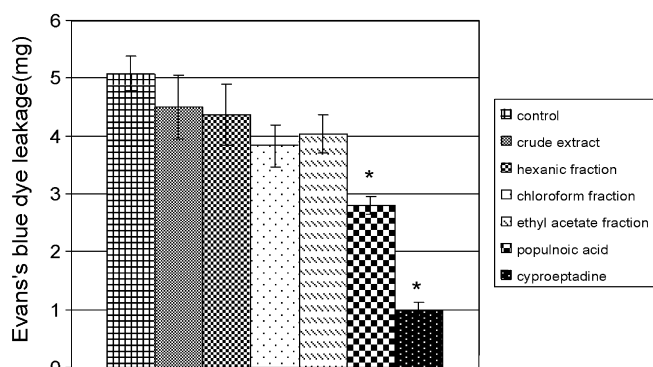


Fig. 4. Effect of the administration of crude extract, hexane, chloroform and ethyl acetate fractions (200 mg/kg), populnoic acid (50 mg/kg) and cyproheptadine (10 mg/kg) on histamine-induced increase in vascular permeability. Each column represents the mean ± S.E.M. of $n=6$. * $p < 0.05$, ANOVA followed by Dunnett's test.

3.4. Histamine-induced increase in vascular permeability

In this assay it was observed a significant activity ($p < 0.05$) only for the groups treated with populnoic acid (50 mg/kg) and cyproheptadine (10 mg/kg) (Fig. 4), which were 44.8% and 80.3%, respectively.

3.5. Granulomatous tissue induction

In this experiment it was observed a significant reduction ($p < 0.05$) only for the groups treated with hexane fraction (200 mg/kg, p.o.) and dexamethasone (0.2 mg/kg, p.o.), daily for 6 days. The reductions were 46.0% and 66.2% for the groups treated with hexane fraction and dexamethasone, respectively (Table 3).

3.6. Analgesic activity

Regarding the writhing test, the crude extract, hexane and chloroform fractions, as well as indomethacin diminished the number of writhings significantly ($p < 0.05$), by 69.6%, 47.2%, 44.8% and 62.8%, respectively (Table 4). On the other hand, none of the assayed plant samples displayed significant results in the hot plate test, but the standard drug (morphine 4 mg/kg, i.p.) increased significantly ($p < 0.05$) the response latency period.

Table 3
Effect of the administration of crude extract, hexane, chloroform and ethyl acetate fractions, populnoic acid and cyproheptadine on cotton pellet-induced granuloma

Groups	Dose (mg/kg)	Mean weight of granuloma (mg)	Inhibition (%)
Control	–	225.52 ± 6.55	–
Crude extract	200	184.02 ± 5.57	18.4
Hexane fraction	200	121.78 ± 7.59*	46.0
Chloroform fraction	200	191.24 ± 8.51	15.2
Ethyl acetate fraction	200	200.03 ± 7.12	11.3
Populnoic acid	50	164.1 ± 8.75	27.2
Dexamethasone	0.2	76.12 ± 3.57*	66.2

The results are expressed as mean ± S.E.M. of $n=6$.

* $p < 0.05$, ANOVA followed by Dunnett's test.

Table 4
Effect of the administration of crude extract, hexane, chloroform and ethyl acetate fractions, populnoic acid and indomethacin on acetic acid-induced abdominal writhing

Groups	Dose (mg/kg)	Number of writhings (mean ± S.E.M.)	Inhibition (%)
Control	–	36.6 ± 4.2	–
Crude extract	200	11.1 ± 4.4*	69.6
Hexane fraction	200	19.3 ± 1.4*	47.2
Chloroform fraction	200	20.2 ± 2.4*	44.8
Ethyl acetate fraction	200	24.6 ± 5.1	32.7
Populnoic acid	50	25.3 ± 3.6	30.8
Indomethacin	10	13.6 ± 4.5*	62.8

The results are expressed as mean ± S.E.M. of $n=8$.

* $p < 0.05$, ANOVA followed by Dunnett's test.

4. Discussion and conclusions

The inflammation is a complex process, which is frequently associated with pain and involves several events, such as: the increase of vascular permeability, increase of granulocytes and mononuclear cells migration, as well as the granulomatous tissue proliferation.

Anti-inflammatory compounds can act on many steps of pathophysiological process. For example, a compound might block the biosynthesis of proinflammatory mediators by direct interaction with a key enzyme (e.g. inhibition of COX-2) or by decreasing enzyme expression (e.g., steroidal anti-inflammatory

compounds) or by reducing substrate levels (e.g., decrease of arachidonic acid releasing). In addition, a compound can either act by inhibiting the release of preformed mediators (e.g., histamine release) or by blocking mediator receptor interaction on target cells (e.g., histamine receptor antagonists). An anti-inflammatory compound may also act by immunostimulation (e.g., maturation of myeloid cells or stimulation of phagocytosis) that in turn promotes an increase removal of the insulting signal molecules, which results in a less aggressive inflammatory response to allergen challenge (Safaihy and Sailer, 1997).

Several experimental protocols of inflammation and pain are used for evaluating the potency of drugs. In the present study, the evaluation of anti-inflammatory and analgesic effects was undertaken using different animal models to fully investigate the potential of *Austroplenckia populnea* to be used in the treatment of inflammatory disorders.

Using the carrageenan-induced paw oedema, which is the most widely used primary test for the screening of new anti-inflammatory agents (Winter et al., 1962), the ED₅₀ was determined as 200 mg/kg. Therefore, this dose was selected to be used in all the experiments, in which crude extract and its fractions were used. To run the assays with the isolated compound, populnoic acid, the dose of 50 mg/kg was chosen based on the similar works published in the literature for pure compounds, and it corresponded to one-fourth of the ED₅₀ obtained for the extract. Also, Carrageenan-induced oedema is an experimental animal model for acute inflammation and it is believed to be biphasic. The early phase (1–2 h) of this assay is mainly mediated by histamine, serotonin and an increasing synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release (Brito and Antonio, 1998).

Prostaglandins (PGs) are metabolites of arachidonic acid, which are synthesized and released by most cell types, and cyclooxygenase (COX) enzymes catalyze the first steps in the biosynthesis of PGs (Teather et al., 2002). COX-2 is often referred to as the inducible isoform of COX, since levels of COX-2 increase in response to several forms of stimulus, including inflammation process in different types of tissue (Teather et al., 2002). In contrast, COX-1 which is the constitutive form of COX, appears to be involved in housekeeping cellular function (Herschman, 1996). Also, another COX isoform was described (COX-3) in brain cortex and heart tissue (Chandrasekharan et al., 2002).

The obtained results indicated that *Austroplenckia populnea* crude extract, and its hexane and chloroform fractions inhibited significantly the formation of the carrageenan-induced rat paw oedema, measured in the third hour of experiment (peak of oedema formation).

Phytochemical analyses of the *Austroplenckia populnea* extract and its fractions indicated that the large majority of its constituents were triterpenes. In this regard, the anti-inflammatory activity is a common property of many triterpenoids (Safaihy and Sailer, 1997). The anti-inflammatory effects of triterpenes (i.e. oleanolic and ursolic acids) have been attributed to various mechanisms including: inhibition of lipoxygenase and cyclooxygenase activities, inhibition of elastase and inhibition of complement activity, possibly through the inhi-

bition on C3-convertase of the classical complement pathway (Singh et al., 1992).

Also, it is well known that inflammation sites present a high concentration of free radicals and oxidants, which play an important role in different inflammation process. Therefore, anti-oxidant compounds can be helpful to avoid this process (Salvemini et al., 1996).

The antioxidant activity has been described for several triterpenes, such as: α - and β -amirins, oleanolic acid, ursolic acid, leupeol and glycirretinic acid, among other related compounds (Andrikopoulos et al., 2003). Phytochemical analyses of the *Austroplenckia populnea* extract and its fractions indicated that the large majority of its constituents were triterpenes. Hence, the anti-inflammatory activity of the extract and its fractions may be partially devoid to its relative antioxidant activity along with the inhibition of important enzymes of the inflammatory response.

Regarding the dextran-induced rat paw oedema, it was observed a significant reduction of oedema in all tested groups. On the other hand, for the histamine assay, only the group treated with populnoic acid displayed significant reduction of oedema.

It is well established that carrageenan and dextran induce rat paw oedema by different mechanisms. Dextran is a polysaccharide of high molecular weight that induces anaphylactic reaction after injection in mice and rats extremities, which is characterized by extravasation and oedema formation, as consequence of liberation of histamine and serotonin from mast cells (Van Wauve and Goossens, 1989). The histamine is a basic amine related with inflammatory and allergic process causing, among several effects, both vasodilatation and increase of vascular permeability (Rang et al., 2001). In this work, on the light of the obtained results, it is suggested an antihistaminic activity. However, neither crude extract nor its fractions were active in inhibiting histamine-induced rat paw oedema, but on the other hand, activity was observed on the dextran-induced rat paw oedema assay. Therefore, considering that histamine is the main mediator in both models, it can be suggested that the crude extract and its fractions contain compounds that are capable of inhibiting histamine liberation from mast cells, but not from tissue release. Moreover, populnoic acid displayed inhibition activity in both models, allowing to suggest that this compound may inhibit histamine release from mast and/or block histamine-receptors.

Regarding the histamine-induced increase in vascular permeability assay, it was observed a significant decreased in dye leakage only in the group treated with populnoic acid. This result ratifies the one obtained in the histamine-induced rat paw oedema assay. Besides, diminution in vascular permeability was described for the triterpenes α - and β -amirins (Oliveira et al., 2004).

The granulomatous tissue induction is a widely used method for assessment of anti-inflammatory compounds on chronic inflammation, and anti-inflammatory steroidal drugs show higher activity in this model (Olajide et al., 2000). Among the assayed samples, only the hexane fraction displayed a significant reduction in the cotton pellet-induced granuloma assay. Considering that *Austroplenckia populnea* bears high concentration of triterpenes and steroids, and that hexane is a good solvent to

extract both class of compounds, this result would be expected for the hexane fraction.

Regarding the analgesic activity of the assayed samples on the mice writhing assay, it is known that the intraperitoneal administration of agents that irritate serous membranes, such as acetic acid, provokes a stereotypical behavior in mice characterized by abdominal contractions, movements of the body as a whole, twisting of dorsoabdominal muscles, and a reduction in motor activity and coordination (Bars et al., 2001). The quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats obtained after the intraperitoneal injection of acetic acid demonstrated high levels of prostaglandins PGE₂α and PGF₂α during 30 min after stimulus (Deraedt et al., 1980). Studies conducted by Seito et al. (2002) showed that hexane and methanol extracts of *Austroplenckia populnea* decreased the abdominal pain in the acid acetic induced writhing assay in mice using high doses (1 g/kg).

Although, the abdominal constriction response induced by acetic acid is a very sensitive procedure that enables the detection of peripheral antinociceptive activity of compounds using animal protocols, which is not a specific model (Takahashi and Paz, 1987). This model involves different nociceptive mechanisms, such as sympathetic system (biogenic amines release), cyclooxygenases and their metabolites (Duarte et al., 1988) and opioid mechanisms (Collier et al., 1968). Because of that, the hot plate test was carried out aiming to evaluate whether the *Austroplenckia populnea* compounds could show any central analgesic effect, but none sample was active. Thus, the assayed samples did not display any analgesic effect on the central nervous system that could contribute to its peripheral analgesic effects.

It is important to point out that extracts of the *Austroplenckia populnea* were reported to display antiulcer activity (Seito et al., 2002). Therefore, the combination of the anti-inflammatory and gastroprotective effects in the same plant extract should be taken into account, because of the serious limitations of a large number of anti-inflammatory agents, that produce gastric irritation, bleeding and mucosal cellular damage.

Considering that there are only a few preliminary data reported in the literature regarding the anti-inflammatory properties of *Austroplenckia populnea* preparations, and that it has been largely used in folk medicine to treat inflammatory disorders, mainly rheumatism, the obtained results corroborate the folkloric use of this plant. In addition, considering that extracts of this plant also display gastroprotective effect, as reported by literature, this plant would be a good candidate for further development of a new phytotherapeutic medicine. Also, the obtained results in this work contribute significantly to the pharmacological validation for the safe use of *Austroplenckia populnea* preparations.

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