

Antiulcerogenic Activity of Crude Extract, Fractions and Populnoic Acid Isolated from *Austroplenckia populnea* (Celastraceae)

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Many plant crude extracts and their isolated compounds are the most attractive sources of new drugs and show promising results for the treatment of gastric ulcers. *Austroplenckia populnea* is commonly known as “marmelinho-do campo, mangabeira-brava, mangabarana and vime” and it has been used in folk medicine as anti-dysenteric and anti-rheumatic. Powdered bark wood (3.25 kg) was macerated with aqueous ethanol (96%) and the extract was concentrated under reduced pressure to yield 406 g of crude hydralcoholic extract. The hydralcoholic extract was suspended in aqueous methanol and partitioned with hexane, chloroform and ethyl acetate (EtOAc) in sequence, yielding 8.0 g, 9.5 g and 98.17 g of crude extracts, respectively. Chromatography of the hexane extract over a silica gel column led to the isolation of the triterpene populnoic acid. The oral administration of hydralcoholic, hexane, chloroform and EtOAc extracts (200 mg/kg) decreased the ulcer lesion index (ULI) by 83.15%, 46.87%, 32.2%, 68.12%, respectively. Oral administration of populnoic acid (100 mg/kg) diminished the ULI by 55.29%. All the obtained results were significant in comparison with the negative control, with exception of the chloroform extract.

Key words: *Austroplenckia populnea*, Gastric Ulcer, Populnoic Acid, Antiulcerogenic Activity

Introduction

Many plants and their active compounds have been used as therapeutic agents (Volgelzang, 2001). Gastric ulcer, one of the most widespread health problems, is believed to be due to an imbalance between acid and pepsin, along with weakness of the mucosal barrier (Alkofahi and Atta, 1999).

The current medicinal treatment of peptic ulcer is generally based on the inhibition of gastric acid secretion by H₂-blockers, such as omeprazole and antimuscarinics, as well as on the acid-independent therapy provided by sucralfate and bismuth (Barocelli *et al.*, 1997). Although, there are many products used for the treatment of gastric ulcers, most of these drugs produce several adverse reactions (Ariyphisi *et al.*, 1986). However, either plant crude extracts or their pure compounds are good sources for the development of new drugs and have been shown to produce promising results in the treatment of gastric ulcers (Hiruma-Lima *et*

al., 2001). There are many works reporting the antiulcerogenic effect of both plant extracts and their pure compounds, such as: Perera *et al.* (2001) on *Rhizophora mangle* (500 mg/kg), Suffredini *et al.* (1999) on *Microgramma squamulosa* (400 mg/kg), Markman *et al.* (2004) on *Campomanesia xanthocarpa* (400 mg/kg), Rodriguez *et al.* (2003) on oleonic acid (25, 50 and 100 mg/kg) isolated from *Fabiana imbricata*, and Jorge *et al.* (2004) on *Maytenus ilicifolia* (320 mg/kg).

Austroplenckia populnea (Reiss) Lundell is a Brazilian plant, which belongs to the Celastraceae family and occurs in “cerrado” (savanna) vegetation, mainly in the States of São Paulo, Minas Gerais and Goiás. This botanical family includes several species that have been widely used in folk medicine for their antiulcerogenic, analgesic, male antifertility, anti-inflammatory and other activities. *A. populnea* is commonly known as “marmelinho-do campo, mangabeira-brava, mangabarana and vime” and it is a folk medicine used as both anti-

dysenteric and anti-rheumatic (Côrrea, 1985). Seito *et al.* (2002) reported the antiulcerogenic activity of the *A. populnea* leaves crude hexane extract in mice, using doses ranging from 445 to 2250 mg/kg. Also, Mazaró *et al.* (2000) reported the decrease in sperm number after treatment of rats with 1000 mg/kg of the same extract. A phytochemical study of the *A. populnea* leaf preparations led to the isolation and identification of sesquiterpenes and pentacyclic triterpenes (Vieira-Filho *et al.*, 2000, 2001). In general, pentacyclic triterpenes have been reported to possess anti-inflammatory, antiulcer, antinociceptive and antitumoral properties (Navarrete *et al.*, 2002; Fernandes *et al.*, 2003). The previous report for the antiulcerogenic activity of the crude hexane extract of *A. populnea* was undertaken by using high doses. Therefore, the aim of the present study was to evaluate the antiulcer activity of the *A. populnea* wood bark crude hydralcoholic extract, its hexane, chloroform and ethyl acetate fractions, as well as its pure isolated compound populnoic acid.

Materials and Methods

Plant material and extract preparation

The plant material of *A. 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), where a voucher specimen (n^o. 20415) is deposited at the BOTU herbarium.

The wood bark (3.25 kg) was air-dried at 40 °C, and the dried material was then powdered and exhaustively extracted by maceration with aqueous ethanol (96%), which was concentrated under reduced pressure, yielding 406 g of crude extract. The hydralcoholic (EtOH) crude extract was partitioned between hexane, CHCl₃ and ethyl acetate (EtOAC), yielding 8.0 g, 9.5 g and 98.17 g of crude extracts, respectively.

Gas chromatography analysis of the hexane extract

Chromatographic analysis was carried out with a gas chromatograph (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 film

thickness) was used for all analyses. Hydrogen at a linear gas velocity of 45 cm/s was employed as carrier gas. The 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, Universidade de São Paulo, using both the retention times and the co-injection of the standards with the unknown samples to identify the compounds.

Populnoic acid isolation

The hexane extract (7.0 g) was submitted to repeated column chromatography over 500 g of silica gel (9 × 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. Its purity, which was estimated to be higher than 95%, was determined by TLC analysis using different solvent systems and by ¹³C NMR. Analysis of both ¹H and ¹³C NMR spectra, in comparison with the data published by Itokawa *et al.* (1991), allowed to propose the structure of populnoic acid for the isolated compound.

Animals

Male Wistar rats, weighing 200–250 g, 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*. 12 h 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.

Sample preparation

All the tested samples were dissolved in 1% Tween-80 aqueous solution and administered by gavage. The crude extracts, populnoic acid and cimetidine, a standard drug for this assay, were administered at the doses of 200, 100 and 50 mg/kg, respectively. These doses were similar to those employed in a previous study by Andrade (2005).

Acute gastric ulcer induced by stress

The method described by Basile *et al.* (1990) was employed in this assay. Groups of five animals were treated as described above. 30 min later, each animal was kept for 17 h in a contensor tube, which was immersed vertically until the water reached the neck region of the animal in a tank with current water at 25 °C. After that, the rats were sacrificed by CO₂ inhalation. The stomachs were removed, opened along the greater curvature. The stomachs were gently rinsed with water to remove gastric contents and blood clots and later scanned. The images obtained were analyzed by specific software "EARP" (powered by Vek-Soft, www.veksoft.cjb.net) for measuring each lesion point. The ulcers were classified as: level I, ulcer area < 1 mm²; level II, ulcer area 1–3 mm²; and level III, ulcer area > 3 mm². The ulcerative lesion index (IU) was calculated for each stomach as 1 × (number of ulcers level I) + 2 × (number of

ulcers level II) + 3 × (number of ulcers level III). The curative ratio was determined as follows: %C = 100 – (IU_{treated} × 100 / IU_{control}), where IU_{treated} and IU_{control} are according to the values in Table I (lesion index).

Statistical analysis

Data are reported as mean ± S.E.M. and were statistically analyzed by analysis of variance, with differences between means at the *p* < 0.05 level being determined by Tukey contrast analysis (Sokal and Rohlf, 1995).

Results

The GC analysis of the hexane extract of *A. populnea* led to the identification of the triterpenes epitaraxerol, β-amirin, lupeol, lupeol acetate, β-friedelanol, friedelin and populnoic acid (Fig. 1), which was also isolated by chromatographic means, as well as the steroids stigmasterol, campesterol and β-sitosterol.

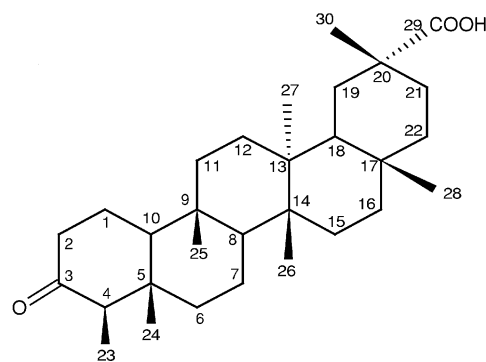


Fig. 1. Structure of populnoic acid.

Table I. Antiulcerogenic activity in rats of hydralcoholic (EE), hexane (HE), chloroform (CE) and ethyl acetate (AE) extracts of the bark wood of *A. populnea* and populnoic acid.

Treatment	Dose [mg/kg]	<i>n</i>	Level I	Level II	Level III	Lesion index	Curative ratio (%)
Control		5	31.40 ± 4.90	16.00 ± 3.10	9.60 ± 0.80	21.61	–
EE	200	5	3.60 ± 0.95*	2.8 ± 0.96*	3.02 ± 0.94	3.64*	83.15
HE	200	5	19.05 ± 2.04	10.8 ± 2.63*	5.6 ± 1.55*	11.48*	46.87
CE	200	5	23.52 ± 3.25	11.8 ± 1.15	8.82 ± 1.24	14.69	32.20
AE	200	5	9.40 ± 2.31*	5.25 ± 0.58*	4.60 ± 1.12*	6.88*	68.12
Populnoic acid	100	5	16.25 ± 0.53*	8.50 ± 2.66	5.15 ± 1.64	9.66*	55.29
Cimetidine	100	5	18.33 ± 2.48*	8.05 ± 0.77*	3.33 ± 0.55*	10.4*	51.87

Level I, ulcer area < 1 mm²; level II, ulcer area 1–3 mm²; level III, ulcer area > 3 mm².

* Significant compared to control: *p* < 0.05.

The oral administration of hydralcoholic, hexane, chloroform and EtOAc extracts (200 mg/kg) reduced the ulcer lesion index (ULI) by 83.15%, 46.87%, 32.2% and 68.12%, respectively. Oral administration of populnoic acid (100 mg/kg) diminished the ULI by 55.29%, and cimetidine (*N*-cyano-*N*-methyl-*N'*-[2-[[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]-ethyl]-guanidine) at 100 mg/kg reduced the ULI by 51.87%, as well. All the obtained results were significant in comparison with the negative control, with exception of the chloroform extract. The effects of different extracts and populnoic acid are displayed in Table I.

Discussion

The higher plants are well known as a promising source for the discovery of new leading pharmaceuticals. In this regard, there are many plant extracts used in folk medicine for the treatment of gastric ulcer. There are several experimental models for evaluating antiulcer activity either for plant crude extracts or pure natural and synthetic compounds (Borrelli and Izzo, 2000).

Austroplenckia populnea was selected for this work based not only on the previous published work on antiulcer activity (Seito, 2002), but also because it belongs to the Celastraceae family, from which *Maytenus ilicifolia* stands out as a commercial phytotherapeutic for the treatment of gastric ulcer (Souza-Formigoni *et al.*, 1991). Moreover, it was found that the triterpenes are the major compounds responsible for the *M. ilicifolia* antiulcer activity (Vilegas and Lanças, 1994). In this regard, the reported results in this work corroborate the results obtained by Vilegas and Lanças (1994), since the crude extracts of *A. populnea*, as well as the pure isolated compound, populnoic acid, displayed an antiulcer activity similar to the one obtained for cimetidine. In addition, the displayed activity was obtained by using a dose lower than the one reported for the crude extracts of *Maytenus ilicifolia* (Jorge *et al.*, 2004). Nevertheless, Queiroga *et al.* (2000) reported that two isolated triterpenes from *M. ilicifolia* were inactive in the antiulcer activity assay. However, it is known that the crude extracts of *M. ilicifolia* displayed good antiulcer activity (Jorge *et al.*, 2004). In this work it was found that the crude hydralcoholic extract of *A. populnea* displayed an antiulcer activity higher than the one obtained for the pure compound populnoic acid, which also displayed a good activity. Therefore, there might be a synergistic ef-

fect among the triterpenes present in these crude extracts, allowing the use of these plants only as phytotherapeutic crude preparations for the treatment of gastric ulcer. Moreover, previous studies have shown that some terpenes or their derivatives, isolated from higher plants, display *in vivo* antiulcerogenic activity (Rodriguez *et al.*, 2002, 2003; Lewis and Hanson, 1991). The active compounds, comprising sesquiterpenes, diterpenes and triterpenes seem to act by different and complementary mechanisms, exhibiting as common feature an improvement of the mucosal defensive factors more than the effects on the aggressive factors (Hiruma-Lima *et al.*, 1999).

The chosen method to induce ulcer described by Basile *et al.* (1990) led to extensive ulcer and petechial lesions formation in the negative control group, making this technique suitable for investigation of antiulcer products. Besides, it was found that this method provides a more homogeneous response among the individual animals in comparison with other protocols (Basile *et al.*, 1990).

There are several factors that may induce ulcer in human beings, such as: stress, chronic use of anti-inflammatory drugs and continuous ingestion of alcoholic beverages, among others (Barocelli *et al.*, 1997). Gastric stress ulceration is probably mediated by the release of histamine, which not only enhances the acid secretion but also reduces mucous production. Moreover, stress induced ulcer in animal models may be partially or entirely prevented by vagotomy, since the increased vagal activity has been suggested to be the main factor in stress induced ulceration (Singh and Majumdar, 1999).

The candidate for an effective drug against peptic ulcer should basically act either by reducing the aggressive factors on gastro-duodenal mucosa or by increasing mucosal resistance against them (Larach and Malagelada, 1982).

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