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Characterization and biological properties of *Pouteria torta* extracts: a preliminary study

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ABSTRACT: Hexane, ethanol and aqueous extracts and fractions from leaves of *Pouteria torta* (Mart.) Radlk (Sapotaceae), a perennial tree, widespread in the Brazilian Cerrado, were tested for cytotoxicity with the *Artemia salina* toxicity model. Only the aqueous crude extract and the MeCN: $CHCl_3$ fraction of the ethanol extract presented toxicity (0.28 mg/mL and 0.27mg/mL, respectively). Lupeol acetate was isolated from the hexane extract. It is the first report of lupeol acetate from the genus *Pouteria*.

Keywords: *Pouteria torta*, *Artemia salina*, Sapotaceae, lupeol acetate.

INTRODUCTION

Pouteria torta (Mart.) Radlk (Sapotaceae), popularly called "guapeva", "curiola", "acá ferro", "abiu do cerrado", and "grão de galo", is a perennial tree widespread in the Brazilian Cerrado, but can also be found from the Amazon region to the State of Bahia. People living in Cerrado eat the yellow fruits and use the tree bark as an antidysenteric medicine.

In previous studies about the chemical composition of the hexane and dichloromethane extracts from the flowers and fruits of *P. torta*, fat acid mixtures, poliisoprenoid compounds, hydrocarbon mixtures, and triterpenes were obtained (David, 1993).

Recently, it was reported that the methanol extract of *P. torta* leaves showed antimicrobial activity against *Cladosporium sphaerospermum*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Pseudomonas aeruginosa* (Alves et al., 2000).

As shown above, despite the therapeutic properties attributed to *P. torta*, the plant has not been well investigated for its biological activities, and few data can be found regarding to the chemical composition of this species. The main purpose of this study was to evaluate the potential antitumor properties of the hexane, ethanol, and aqueous extracts and fractions from the leaves of *P. torta* by using the *Artemia salina* toxicity model, as a pre-screening procedure, and the structural characterization of one of the components from the hexane crude extract.

MATERIAL AND METHODS

General procedures

Column chromatography was carried out using silica gel Merck 60 (0.063- 0.200 mm). Analytical thin layer chromatography was performed on precoated ALUGRAM Sil G Machery-Nagel silica gel (60/0.2 mm) plates using UV light, anisaldehyde/H_2SO_4, and vanillin/H_2SO_4 reagents (Wagner; Bladt, 1996) to visualize the spots. ^{13}C-NMR spectra were recorded on a Mercury plus spectrometer, Varian (7.05 T), operating at 75 MHz and performed in $CDCl_3$/TMS. ^{13}C chemical shifts (δ) are reported in parts per million (ppm) relative to TMS.

Plant material

Leaves of *P. torta* were collected on the campus of the Universidade de Brasília in July, 2003. An exsiccate is deposited in the Herbarium of the Universidade de Brasília (UB) and the species was identified by Professor J. E. de Paula (voucher number JElias de Paula 3674).

Extraction

The plant material was dried at room temperature and powdered in a knife mill. Part of this material (918 g) was macerated at room temperature for seven days (repeated for three times), first with hexane, followed by ethanol. After filtration, the solvents were removed under reduced pressure, at temperatures below 40 °C.

The aqueous extract, from 400 g of the powdered material, was obtained by infusion, using distillated water (3 L). After filtration, the water was removed by lyophilization technique to yield 44 g (11 %) of crude aqueous extract.

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Table 1. Toxicity screening of *Pouteria torta* crude extracts and fractions against *Artemia salina* larvae.

Samples	LD_{50} (ppm)	LD_{50} (mg/mL)	Confidence interval 95%
Hexane crude extract	>1000	>1.0	-
Ethanol crude extract	>1000	>1.0	-
Aqueous crude extract	280	0.28	154 - 498
MeCN:$CHCl_3$ fraction	270	0.27	112 - 427
Aqueous fr. fraction	>1000	>1.0	-
Hexane fr. fraction	>1000	>1.0	-
Lupeol acetate (Pt1)	>200	>0.2	-
Potassium dichromate	80	0.08	53 - 85

Finally, the ethanol extract was partitioned using Hexane: $CHCl_3$: CH_3CN: H_2O (2:1: 3.4:1) (Duarte et al., 2000) yielding three fractions. The yields of crude extracts and fractions are presented in Figure 1.

Brine shrimp lethality test (BST)

Table 2. Comparison of Pt1 ^{13}C NMR chemical shifts ($CDCl_3$, 75 MHz) with literature data of the lupeol acetate.

C	Pt 1 (δ)	Lupeol acetate
		Sholichin (1980)
1	38.4	38.4
2	23.6	23.7
3	80.9	81.0
4	37.7	37.8
5	55.3	55.4
6	18.1	18.2
7	34.3	34.3
8	40.9	40.9
9	50.3	50.4
10	37.1	37.1
11	20.9	21.0
12	25.0	25.1
13	38.0	38.1
14	42.9	42.9
15	27.5	27.5
16	35.5	35.6
17	43.0	43.0
18	48.2	48.3
19	48.0	48.0
20	150.9	150.9
21	29.8	29.9
22	40.0	40.0
23	28.0	28.0
24	16.5	16.5
25	16.1	16.2
26	16.0	16.0
27	14.4	14.5
28	18.1	18.0
29	109.3	109.4
30	19.2	19.3
$\underline{C}H_3CO$	21.3	21.3
$CH_3\underline{C}O$	171.0	170.8

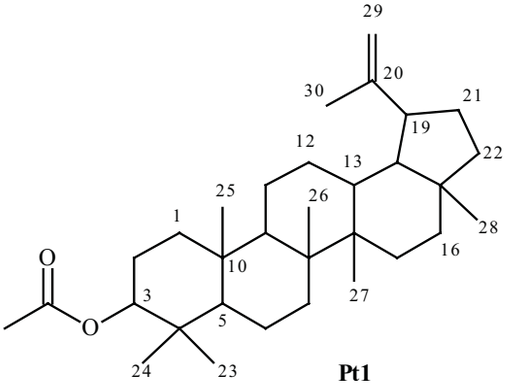
All fractions were tested for brine shrimp lethality. The assay was performed basically according to the simplified Meyer's method (Meyer et al., 1982). Briefly, brine shrimp, *Artemia salina* L. encysted eggs (Maramar) were incubated in artificial seawater at 28 °C. Samples were dissolved in 200 µL of DMSO plus 20 mL of artificial seawater. Serial dilutions (triplicate) were prepared in the same solution. Metanauplii (10 units) was added to each set of tubes containing samples and the cultures further incubated for 24 h. Controls containing DMSO were included on each set of experiments. Potassium dichromate was used as reference standard. LD_{50} (after 24 h) were calculated by Probit analysis.

Chemical constituents of hexane extract

Hexane extract (5 g) was chromatographed over silica gel, by using hexane: ethyl acetate: methanol gradient. After analysis by TLC, the obtained 24 fractions were gathered in 8 groups. Group 1 (93 mg) furnished a hydrocarbon mixture. Group 3 (50 mg) was extracted by acetone, furnishing 25 mg of amorphous white solid characterized as long chain esters mixture. Groups 5, 6, 7 and 8 (503 mg) were extracted by acetone: methanol (1:1) furnishing 82 mg (1.2 %) of a white amorphous solid (Pt1). Groups 2 and 4 were found to be very complex mixtures and were not studied at this time.

RESULTS AND DISCUSSION

The study of the leaves of *Pouteria torta* allowed to obtain three crude extracts and by the tri-phase



Pt1

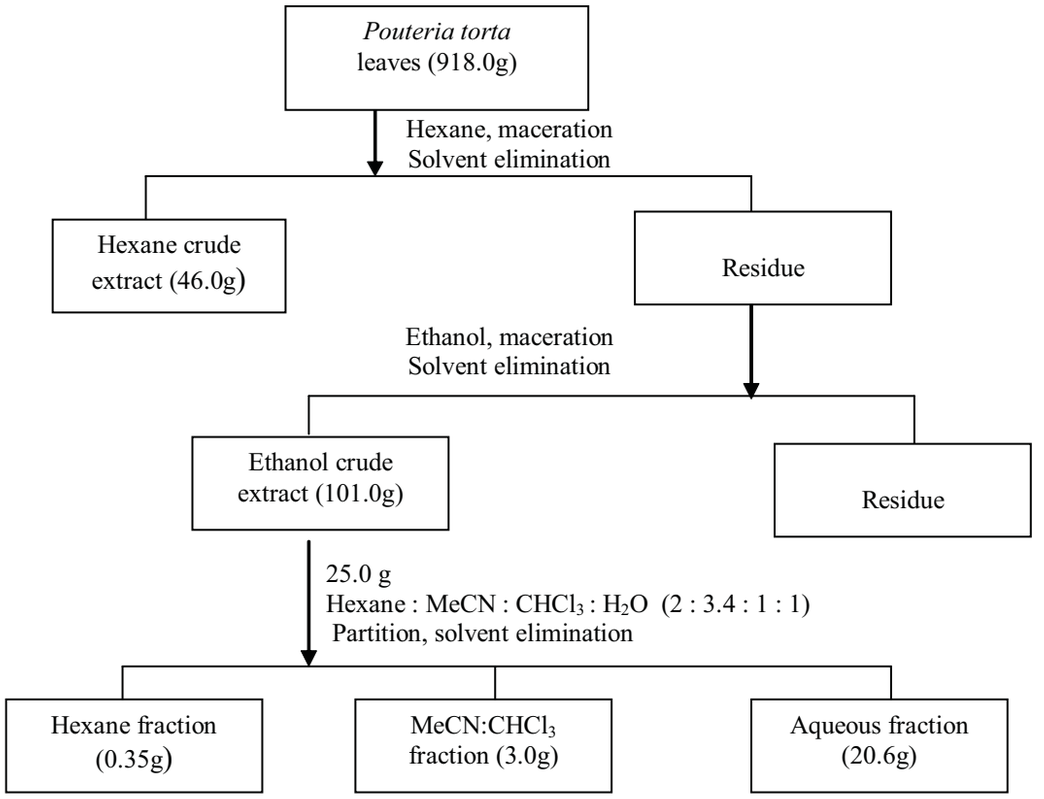


Figure 1. Crude extracts and fractions from *Pouteria torta* leaves

partition, we could separate the ethanol extract in three fractions with different polarity.

The chromatographic separation of the hexane extract of *P. torta* on silica gel column afforded Pt1. The ^{13}C NMR spectrum of Pt1 showed signals which led to its identification as lupeol acetate by comparison of NMR chemical shifts found at the literature data (Sholichin et al., 1980; Freire et al., 2002). Herein, we point out the fact that this is the first report of lupeol acetate in *Pouteria* species.

BST results are presented at Table 1. In the toxicity evaluation of plant extracts by brine shrimp bioassay, a LD_{50} value lower than 1mg/mL is considered bioactive (Meyer et al. 1982). In our experiments, we have used Meyer classification to the crude extracts and fractions, but we assume pure compounds toxic, when the LD_{50} value is lower to 0.2 mg/mL. Based on this classification, aqueous crude extract and acetonitrile: chloroform fraction were considered toxic to *Artemia salina* larvae (0.28 mg/mL and 0.27 mg/mL, respectively), and lupeol acetate was considered non-toxic. Taking into account the good correlation between the toxicity on *Artemia salina* with that on tumor cell lines (e.g, KB, P-388, L5178Y and L1210) (De Rosa et al., 1994), as well with several others assays such as trypanocidal (Zani et al., 1995) and phototoxicity (Ojala et al., 1999), besides to seeking compounds for protective activity against active oxygen species (AOS)-related damage (Mathews, 1995), further studies on *Pouteria torta* will be carried out to better define the spectrum of its biological activity and to understand the chemical composition of its extracts.

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