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Triterpenes from *Pouteria gardneri* (Mart. & Miq.) Baehni extracts

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ABSTRACT

Pouteria gardneri (Sapotaceae) is a South America native species found in Bolivia, Paraguay, and Brazil. Local inhabitants appreciate the edible fruits ("sapotinha"). In Brazil, this species grows in almost all biomes, including the Cerrado. However, there are few studies about this plant. In a phytochemical study of leaves of *P. gardneri*, the triterpenes β-amyrin, α- and β-amyrin acetate, lupeol acetate, ψ-taraxasterol acetate were characterized in hexane extract, by GC-MS and ^{1}H and ^{13}C NMR analysis. Further column chromatography separation led to the isolation of α-amyrin acetate. Ethanol crude extract was submitted to a triphase liquid-liquid partition. From silica gel column of MeCN-$CHCl_3$ fraction, a mixture of oleanolic and ursolic acid was obtained. All isolated substances were characterized by ^{1}H and ^{13}C spectra data, in comparison to literature. As far we know, it is the first time the chemistry composition of *Pouteria gardneri* is investigated.

INTRODUCTION

Sapotaceae is a pantropical family of flowering plants (Alves-Araújo and Alves, 2013). In Brazil, there are 12 genera and about 230 known species (Carneiro *et al.*, 2015). *Pouteria* is the largest genus of Sapotaceae (Alves-Araújo *et al.*, 2014). Although 122 species had been recorded in Brazil (Alves-Araujo, 2015), only a few species were evaluated for the chemistry composition. It was observed that triterpene and steroidal compounds mean to be quite common in this genus (Anjaneyulu, 1965; Ardon and Nakano, 1973; Che *et al.*, 1980; Pellicciari *et al.*, 1972; Perfeito *et al.*, 2005; Silva *et al.*, 2009). From polyphenol compounds, myricitrin is, usually, found in *Pouteria* species and maybe can be considered a *Pouteria* chemical marker (Lim, 2013; Ma *et al.*, 2003; Ma *et al.*, 2004). *Pouteria gardneri* (Mart. & Miq.) Baehni is one of the Brazilian native species, and is commonly known as "cabritão", "frutinha-de-veado", "sapotinha", "leiteiro-preto", or "leiteiro-da-folha-miuda" (Alves-Araújo and Alves, 2013; Coelho *et al.*, 2009a; Mesquita *et al.*, 2005). This species can be found in almost all Brazilian biomes, including Cerrado (Alves-Araujo *et al.*, 2016). Also, *P. gardneri* can be found in Bolivia and Paraguay (TROPICOS, 2014). The wood is used to build houses, and the small fruits are eaten freshly (Paula *et al.*, 2011). Extracts from *P. gardneri* were evaluated about trypanocidal, antimalarial and anti-leishmania activities and did not present activity (Mesquita *et al.*, 2005; Mesquita *et al.*, 2007). The same researchers evaluated *P. gardneri* extract about cytotoxicity against four cell lines (SF-295, HCT-8, MDA-MB-435, and HL-60) and no activity was found (Mesquita *et al.*, 2009).

Also, several extracts from this species were tested for toxicity to *Dipetalogaster maxima* and *Aedes aegypti* larvae and did not present significant activity (Coelho *et al.*, 2009a; Coelho *et al.*, 2009b). Extracts from leaves of *P. gardneri* also were evaluated for potential enzyme inhibition. The extracts did not show inhibition of tyrosinase (Souza *et al.*, 2012a). However, the ethanol and hexane crude extracts presented α-glucosidase (100 and 95%, respectively) and α-amylase (71 and 31 %, respectively) inhibition (Souza *et al.*, 2012b).

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Therefore, because there is a lack of knowledge about chemical composition *P. gardneri*, the aim of this work is to report the phytochemical study of hexane and ethanol extracts from leaves.

MATERIAL AND METHODS

All process was monitored by analytical TLC, performed on precoated ALUGRAM sil G Machery-Nagel silica gel (60/0.2 mm) plates, using anisaldehyde reagent (Wagner and Bladt, 1996). ^{1}H and ^{13}C NMR spectra were registered on Varian (7.05 T) Mercury-plus spectrometers, operating at 300 MHz and performed in $CDCl_3$. Offline processing was conducted using Mestre-C NMR Software (Mestrelab Research).

Analytical GC-MS was carried out on a Thermo Finnigan system with chromatograph TRACE GC coupled to mass spectrometer Polaris Q (EI, 70 eV) fitted with HP5MS (5% diphenyl, 95% dimethylpolysiloxane) capillary column (30 m×0.25 mm×0.25 mm). The carrier gas was helium with flow rate 0.6 mL/min. The temperature of the column was programmed from 60 °C (1 min) – 40 °C/min to 140 °C and 4 °C/min to 300 °C (10 min). The injection volume was 0.2 μL solution 1 mg/mL (sample in chloroform). The obtained mass spectra were compared to NIST standard reference database.

Plant material

The leaves of *Pouteria gardneri* were collected at Paranoá - DF, in July 2003 and identified by Professor J. E. de Paula. An exsiccate was deposited at Herbarium of Universidade de Brasília (UB) (voucher number JElias de Paula 3718). The extraction was performed according to previously reported technique (Perfeito *et al.*, 2005).

Briefly, the dried and powdered plant material (141.8 g) was macerated at room temperature for seven days (repeated three times) with hexane. After filtration, the solvent from the extractive solution was eliminated under reduced pressure, at 40 ^{o}C, furnishing 5.3 g (3.7% yield) of crude hexane extract (HG) and 22.9 g (16.2 % yield) of crude ethanol extract (EG).

Isolation procedures

Hexane extract HG (3.0 g) was submitted to filtration over Silica gel 60 Merck, furnishing 5 fractions according to the used eluent: hexane (HGA, 300.0 mg), hexane/ethyl acetate (1:1) (HGB, 2.4 g), ethyl acetate (HGC, 100.0 mg), ethyl acetate/ methanol (1:1) (HGD, 100.0 mg) and methanol (HGE, 0.3 mg). HGB fraction (1.5 g), after column chromatography over silica (Silica gel 60 Merck), using hexane- AcOEt- MeOH gradient, furnished 17 groups of fractions. Group HGB3 (92.9 mg), eluted with Hex: AcOEt (9:1) was a mixture of triterpenes (***1***) and long-chain fatty esters. Further silica gel column chromatography of HGB3 led to the isolation of (***2***) (22 mg).

Ethanol extract EG (3.2 g) was partitioned using Hexane: $CHCl_3$: CH_3CN: H_2O (2:1: 3.4:1), as previously described (Perfeito *et al.*, 2005). The process led to three fractions: hexane (EGF, 37 mg) $CHCl_3$:CH_3CN (EGG, 0.5 g) and aqueous (EGH, 2.6 g). Part of $CHCl_3$:CH_3CN fraction EGG (0.1 g) was submitted to silica gel column using hexane – ethyl acetate gradient. The fractions presenting similar TLC profile were pooled, furnishing 18 groups. Group 3 (eluted by Hex: AcOEt (9:2) gave (***3***).

RESULTS

The obtained compounds were characterized by ^{1}H, and ^{13}C NMR spectra, by comparison with literature data (Mahato and Kundu, 1994; Pellicciari *et al.*, 1972; Sholichin *et al.*, 1980). The analysis of ^{13}C NMR of (***1***) allowed the detection of signals corresponding to acetate of α- and β-amyrin and lupeol (Table 1).

Table 1: Chemical shifts of *1* (δ, $CDCl_3$, 75MHz) in comparison with literature data (δ, $CDCl_3$, 75MHz) (Mahato and Kundu, 1994; Sholichin *et al.*, 1980).

C	*1* (δ)	Lupeol acetate[1]	*1* (δ)	α–amyrin[2]	β–amyrin[2]
1	38.4	38.4	38.4	38.7	38.7
2	23.7	23.7	27.4	27.2	27.3
3	80.9	81.0	80.9	78.3	79.0
4	37.8	37.8	38.4	38.7	38.8
5	55.3	55.4	55.2; 55.3	55.2	55.3
6	18.2	18.2	18.2	18.3	18.5
7	34.3	34.3	32.8	32.9	32.8
8	40.8	40.9	39.9	40.0	38.8
9	50.3	50.4	47.6	47.7	47.7
10	37.1	37.1	36.9; 37.6	36.9	37.6
11	20.9	21.0	23.3; 23.6	23.3	23.6
12	25.0	25.1	124.3; 121.6	124.3	121.8
13	38.0	38.1	139.6; 145.1	139.3	145.1
14	42.9	42.9	42.0; 41.7	42.0	41.8
15	27.4	27.5	28.7; 26.2	28.7	26.2
16	35.5	35.6	26.6; 26.9	26.6	27.0
17	43.0	43.0	33.7; 32.5	33.7	32.5
18	48.2	48.3	59.0; 47.5	58.9	47.4
19	48.0	48.0	39.6; 46.7	39.6	46.9
20	150.9	150.9	39.6; 31.0	39.6	31.1
21	29.8	29.9	31.2; 34.7	31.2	34.8
22	40.0	40.0	41.5; 37.1	41.5	37.2
23	28.0	28.0	28.0	28.1	28.2
24	16.5	16.5	15.5	15.6	15.5
25	16.1	16.2	15.7	15.6	15.6
26	16.0	16.0	16.7; 16.8	16.8	16.9
27	14.5	14.5	23.3; 25.9	23.3	26.0
28	18.0	18.0	28.0; 28.4	28.1	28.4
29	109.3	109.4	17.4; 33.3	17.4	33.3
30	19.2	19.3	21.3; 23.7	21.3	23.7
CH_3CO	21.3	21.3	-	-	-
CH_3CO	171.0	170.8	-	-	-

[1]Sholichin (1980); [2]Mahato & Kundu (1994)

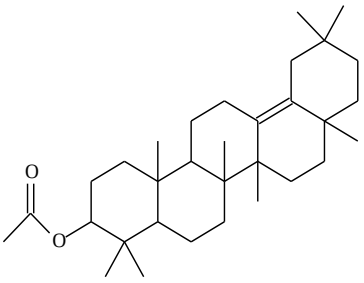
To confirm the presence of acetate of α- and β- amyrin and lupeol in (***1***), a GC-MS analysis was carried out. Based on fragmentation patterns, the analysis showed (***1***), besides the identified compounds, also presented long fatty esters, α-amyrin acetate and ψ-taraxasterol acetate (Table 2). The column chromatography of (***1***), led to the isolation of α-amyrin acetate (***2***) (Table 3).The ^{13}C NMR spectrum of (***3***) also shows the presence of a mixture of two triterpenes. The comparison with literature data led to suggest the presence of ursolic and oleanolic acids (Table 4).

Table 2: Detected triterpenes in hexane extract of *Pouteria gardneri* leaves by GC-MS analysis.

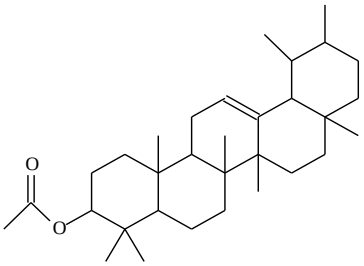
RT (min)	Compound	mass	Main observed fragmentation (m/z)
48.89	β-amyrin	468	426 (M+); 411; 408; 229; 218; 205; 189; 109 (100%); 95
49.19	α-amyrin acetate	468	468 (M); 408; 365; 249; 218 (100%); 203; 189
49.36	ψ-taraxasterol acetate	468	468 (M+); 453; 218; 189 (100)
50.26	β□amyrin acetate	468	468 (M+); 453; 218 (100); 203; 189
50.45	lupeol acetate	468	468 (M+); 453; 408; 189 (100)

Table 3: Chemical shifts of *2* (δ, $CDCl_3$, 75MHz) in comparison with literature data (δ, $CDCl_3$, 75MHz) (Ebajo Jr *et al.*, 2015).

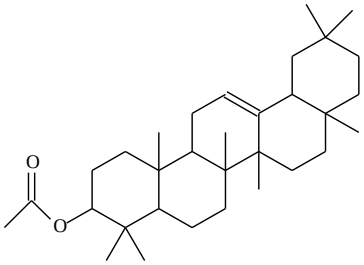
C	*2* (β)	α-amyrin acetate
1	38,4	38,4
2	27,9	27,6
2	28,0	28,0
3	80,6	80,9
4	38,0	37,7
5	55,2	55,2
6	18,2	18,2
7	32,8	32,8
8	40,0	40,0
9	47,5	47,6
10	36,7	36,8
11	23,3	23,4
12	124,3	124,3
13	139,6	139,6
14	42,0	42,05
15	26,9	26,6
16	28,0	28,1
17	33,7	33,7
18	59,0	59,0
19	39,6	39,6
20	39,6	39,6
21	31,2	31,2
22	41,5	41,5
23	28,7	28,7
24	16,8	16,8
25	15,7	15,7
25	16,1	16,1
26	16,8	16,8
27	23,2	23,2
28	28,7	28,7
29	17,4	17,5
30	21,3	21,4
CH_3**C**O	171,0	170,0
CH_3CO	21,3	21,3



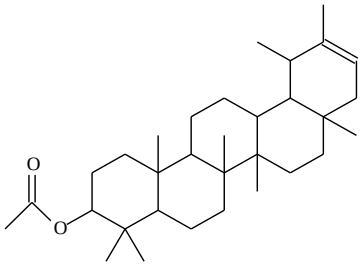
δ-amyrin acetate



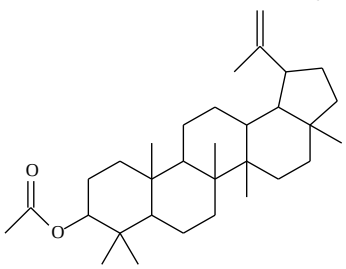
α-amyrin acetate (***2***)



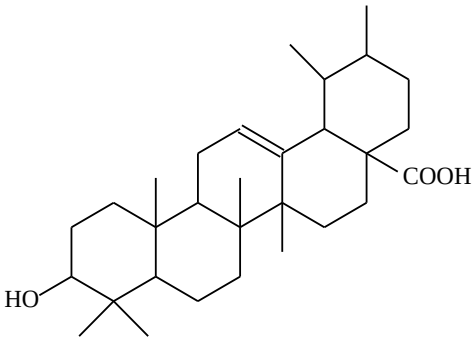
β-amyrin acetate



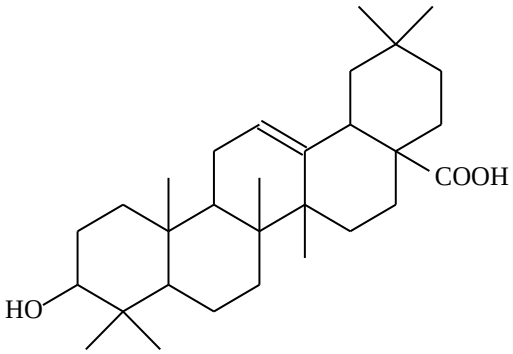
ψ-taraxasterol acetate



lupeol acetate



ursolic acid



oleanolic acid

Table 4: Chemical shifts of *3* (δ, $CDCl_3$, 75 MHz) in comparison with literature data (δ, $CDCl_3$, 50 MHz) (Falcão *et al.*, 2003).

C	*3* (β)	Ursolic acid	*3* (β)	Oleanolic acid
1	38.7	38.8	38.5	38.5
2	27.1	27.3	27.1	27.4
3	79.0	78.8	79.0	78.7
4	38.7	38.8	38.7	38.7
5	55.2	55.4	55.2	55.2
6	18.2	18.4	18.2	18.3
7	32.9	33.0	32.6	32.6
8	39.4	39.6	39.4	39.3
9	47.5	47.5	47.6	47.6
10	37.0	37.0	37.0	37.0
11	23.3	23.3	23.2	23.1
12	125.8	125.5	122.6	122.1
13	137.8	138.0	143.5	143.4
14	41.9	42.0	41.6	41.6
15	28.1	28.2	27.6	27.7
16	24.1	24.3	23.3	23.4
17	47.9	48.1	45.8	46.6
18	52.6	52.8	40.9	41.3
19	39.0	39.1	45.8	45.8
20	38,8	38,8	30.6	30.6
21	30.6	30.7	33.7	33.8
22	36.6	36.7	32.4	32.3
23	28.10	28.2	28.1	28.1
24	15.5	15.5	15.4	15.6
25	15.6	15.7	15.3	15.3
26	17.0	16,9	16.9	16.8
27	23.6	23.6	25.9	26.0
28	182.2	180.0	182.5	181.0
29	17.0	16,9	33.0	33.1
30	21.1	21.2	23.6	23.6

DISCUSSION

The compounds from *P. gardneri* are widespread in higher plants and several biological activities are reported to them. α- and β-Amyrin, as well their derivatives, are quite common in *Pouteria* species and were previously reported to *P. caimito* and *P. tomentosa* (Che *et al.*, 1980; Pellicciari *et al.*, 1972). Moreover, α – and β-amyrin, as well as the compounds α-amyrin acetate, and lupeol acetate were isolated from *P. torta* (Che *et al.*, 1980; Perfeito *et al.*, 2005). However, as far we know, it is the first time the chemical study is reported to *Pouteria gardneri*.

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