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Pentacyclic triterpenes from leaves of Cagaita (*Eugenia dysenterica*)

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ABSTRACT

Phytochemical study of the leaves of *Eugenia dysenterica* led to the identification of five pentacyclic triterpenes: urs-12-en-3β-ol (α-amyrin), olean-12-en-3β-ol (β-amyrin), neolup-12-en-3β-ol (neolupenol), gammacer-16-en-3β-ol and D-friedoolean-14-en-3β-ol (taraxerol). Their identification was based mainly on mass spectra, ^{1}H and ^{13}C NMR. Pentacyclic triterpenes have not been reported before in the literature as constituents of *E. dysenterica*.

INTRODUCTION

Brazil possesses one the richest biodiversity in the world, and Cerrado (Brazilian savannah), one the largest Brazilian biome, presents over 12.000 plant species (Silva *et al.*, 2016), and more than 40% are native. Such characteristics make Cerrado one the most biodiverse savannah (Medeiros *et al.*, 2015). The Myrtaceae is one of the most common family in Cerrado presenting, approximately, 14 genera and 210 species (Conceição and Aragão, 2010). The genus *Eugenia* comprises about 500 species (Cole *et al.*, 2007), from those 379 are native to Brazil, and more than 80 can be found in Cerrado (REFLORA, 2016). One of them is *Eugenia dysenterica* (Mart.) DC, known as "cagaita" or "cagaiteira." Cerrado inhabitants eat the *E. dysenterica* fruits, which also are used by local food industry to prepare ice cream, desserts, jelly and others. A nutritional analysis showed that fruit pulp is rich in vitamins, folates, carotenoids (Cardoso *et al.*, 2011), esters and other compounds (Silva *et al.*, 2015).

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Moreover, this species is widely used for several ethnomedicinal purposes, such as in the treatment of skin and bladder infection (Lima *et al.*, 2011), for the treatment of diarrhea, cardiac diseases, diabetes, jaundice, and the reduction of blood cholesterol levels (Lima *et al.*, 2010; Lima *et al.*, 2011; Silva *et al.*, 2015). Chemical characterization of aqueous, ethanol and methanol extracts from the leaves led to the identification of flavonoids, polyphenols (Prado *et al.*, 2014) and sesquiterpenes (Costa *et al.*, 2000; Duarte *et al.*, 2010; Vilela *et al.*, 2012). However, there is a lack of information about the less polar compounds from this plant species. Therefore, the aim of this work was to carry a phytochemical analysis on the hexane extract from *E. dysenterica* leaves.

MATERIAL AND METHODS

General

Thin-layer chromatography (TLC) analysis was carried out using aluminum-backed silica gel (60F254) plates (Sigma-Aldrich, L × W 20 cm × 20 cm). n-Hexane and ethyl acetate mixture was used as eluent, and the chromatograms were revealed by anisaldehyde– sulphuric acid reagent (5 %).

GC-MS analysis was performed on a Shimadzu gas chromatograph mass spectrometer model QP2010. Compounds were separated on a Rtx-5MS capillary column (30 m x 0.25 mm x 0.25 µm - Restek). Column temperature was programmed from 100 at 320 °C, ranging 5 °C/min with injector temperature at 250 °C and GC-MS interface at 280 °C. Mass detector conditions were: electronic impact (EI) mode at 70 eV; source temperature: 280 °C; scanning rate 0.5 scan/s; mass acquisition range: 40 - 600. Carrier gas was helium at 0.6 mL/min. The obtained mass spectra were compared to NIST standard reference database.

NMR spectroscopic data were recorded on Bruker AscendTM 600 MHz spectrometer with $CDCl_3$ as solvent and TMS as internal standard. Chemical shift values were reported in ppm (δ).

Plant material

Leaves of *Eugenia dysenterica* were collected in June of 2014 at University of Brasilia campus, Brasilia DF (Brazil) and air-dried at room temperature (30 - 35 °C) for ten days. A voucher specimen (UB 914) was deposited in the University of Brasilia Herbarium (UB).

The dried and powdered leaves (1 Kg) were extracted by maceration using hexane as solvent in the ratio 1:6 (sample:solvent, w/v). After filtration, the solvent was eliminated under reduce pressure (Hei- VAP advantage HB/HL/G1, Heidolph, Germany), giving hexane extract (HE, yield 2.2 %).

Isolation procedure

A preliminary fractionation of HE was carried out as previously described (Borges *et al.*, 2016). Briefly, a 16.0 g aliquot of HE was submitted to silica gel 60G Merck filtration (silica layer: high 4.0 cm, diameter 9.7 cm), furnishing five fractions: Fhx (4.1 g); Fhx/AcOEt (11.0 g), FAcOEt (0.6 g), FAcOEt/MeOH (0.1 g); and FMeOH (0.4 g).

A 9.0 g aliquot of the fraction Fhx/AcOEt was chromatographed over silica gel (70-230 µm, Merck) column (diameter: 2.5 cm; high: 90.0 cm), using a gradient of hexane and ethyl acetate as eluent. One hundred subfractions were collected and grouped based on their similar TLC pattern. Subfractions EDD (Hx/AcOEt 9:1, 0.38 g), EDE (Hx/AcOEt 9:1, 0.49 g), EDF (Hx/AcOEt 8:2, 0.44 g), and EDG (Hx/AcOEt 8:2, 0.40 g), were submitted to spectrometric analysis.

RESULTS AND DISCUSSION

Five pentacyclic triterpenes were identified in the hexane extract of *E. dysenterica*: urs-12-en-3β-ol (α-amyrin, 1), olean-12-en-3β-ol (β-amyrin, ***2***), neolup-12-en-3β-ol (neolupenol, ***3***), D-friedolean-14-en-3β-ol (taraxerol, ***4***) and gammacer-16-en-3β-ol (***5***). The obtained compounds were characterized by MS, ^1H and ^{13}C NMR spectra, by comparison with literature data (Mahato and Kundu, 1994; Sagar *et al.*, 2004; Shiojima *et al.*, 1992; Shiojima *et al.*, 1989; Swain *et al.*, 2010). Compounds 1-5 were identified (Figure 1) and assigned with the molecular formula $C_{30}H_{50}O$. The compounds revealed positive respond when sprayed with methanolic – sulphuric acid reagent (5 %). Compounds ***1*** and ***2*** were identified as a mixture in the subfraction EDD with retention time of 51.58 and 51.19 min, respectively. The mass spectrum at EI ionization mode showed a molecular peak at m/z 426.00 and a base peak at m/z 218.10. Spectrometric data are shown below:

α-amyrin, 1. ^1H-NMR ($CDCl_3$, 600 MHz): δ 5.10 (t, J=3.4 Hz, H-12), 0.92 (s, H-23), 0.74 (s, H-24), 0.73 (s, H-25), 0.89 (s, H-26), 1.04 (s, H-27), 0.95 (s, H-28), 0.85 (d, J=5.8, H-29), 0.76 (d, J=6.9, H-30); ^{13}C-NMR ($CDCl_3$, 150 MHz): Results showed in Table 1; MS (m/z): 426 (5.03), 411 (7.09), 393 (1.98), 218 (100.00), 207 (33.25), 203 (40.05), 189 (20.04), 175 (16.13), 135 (21.23). β-amyrin, ***2***. ^1H-NMR ($CDCl_3$, 600 MHz): δ 5.15 (t, J=3.4 Hz, H-12), 0.77 (s, H-23), 0.90 (s, H-24), 0.73 (s, H-25), 0.94 (s, H-26), 1.10 (s, H-27), 1.06 (s, H-28), 0.87 (s, H-29), 0.80 (s, H-30); ^{13}C-NMR ($CDCl_3$, 150 MHz): Results are shown in Table 1; MS (m/z): 426 (5.44), 411 (0.62), 393 (2.38), 218 (100.00), 207 (22.82), 203 (30.01), 189 (33.92), 175 (11.61), 135 (39.64). The Δ^{12}-double bond was identified by signals at δ 124.4 and 139.3 ppm; and 121.7 and 145.2 ppm in the ^{13}C-NMR spectrum, assigned to C-12 and C-13, respectively, of urs-12-en-type and olean-12-ene-type skeletons (Mahato and Kundu, 1994; Shiojima *et al.*, 1992).

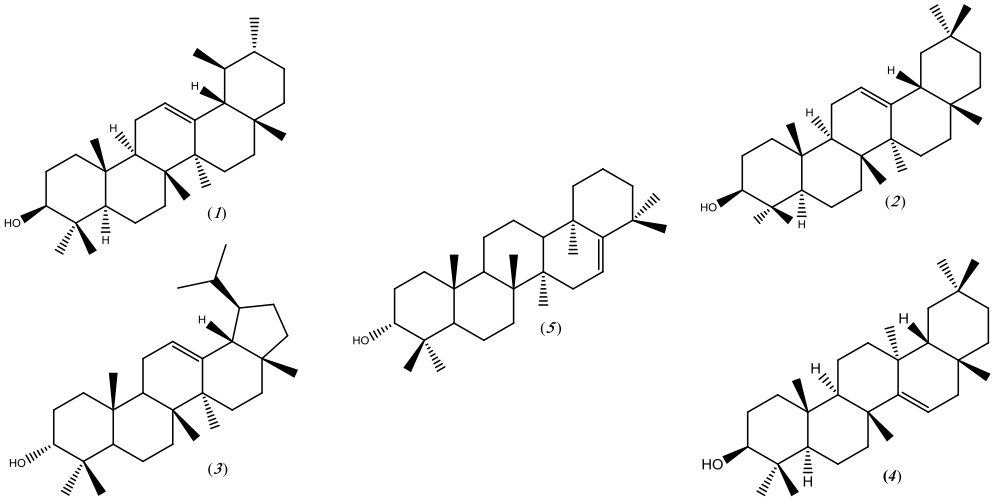

Fig. 1: Pentacyclic triterpenes identified in *E. dysenterica*.

Compound ***3*** was identified in the subfraction EDE with retention time of 51.05 min. The mass spectrum (EI-MS) showed a molecular peak at m/z 426.00 and a base peak at m/z 218.15. Neolupenol, ***3***: ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 3.20 (dd, J=9.8 and 5.1, H-3), 5.15 (t, J=3.7 Hz, H-12), 1.06 (s, H-23), 0.79 (s, H-24), 0.94 (s, H-25), 0.99 (s, H-26), 1.22 (s, H-27), 0.91 (s, H-28), 0.76 (d, J=4.8, H-29), 0.85 (d, J=4.8, H-30); ^{13}C-NMR ($CDCl_3$, 150 MHz): Results are shown in Table 1; MS (m/z):426 (7.51), 411 (13.72), 393 (2.68), 218 (100.00), 207 (18.67), 203 (71.37), 189 (74.56), 175 (22.54), 135 (37.96). The presence of a Δ^{12}-double bond was corroborated by signals at δ 124.4 and 139.6 in the ^{13}C-NMR spectrum, assigned to C-12 and C-13, respectively. Hence on the basis of its all spectral data ***3*** was characterized as Neolupenol (Sagar *et al.,* 2004; Shiojima *et al.,* 1992).

Compound ***4*** was identified in the subfraction EDF with retention time of 50.85 min. The mass spectrum (EI-MS) showed a molecular peak at m/z 426.00 and a base peak at m/z 204.10.

Taraxerol, ***4***: ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 3.19 (m, H-3), 5.52 (dd, J=8.1 and 3.3 Hz, H-15), 1.89 (dd, H-16), 0.97 (s, H-23), 0.90 (s, H-24), 0.79 (s, H-25), 0.89 (s, H-26), 1.10 (s, H-27), 0.80 (s, H-28), 0.92 (s, H-29), 0.89 (s, H-30); ^{13}C-NMR ($CDCl_3$, 150 MHz): Results are shown in Table 1; MS (m/z):426 (6.09), 411 (9.48), 302 (29.78), 287 (49.01), 271 (3.27), 269 (32.04), 257 (19.01), 207 (48.56), 204 (100.00), 189 (91.54), 135 (90.41) e 123 (33.90). The presence of a Δ^{14}-double bond was corroborated by signals at δ 158.1 and 116.9 in the ^{13}C-NMR spectrum, assigned to C-14 and C-15. Thus the compound ***4*** was identified as taraxerol (Shiojima *et al.,* 1992; Swain *et al.,* 2010).

Compound 5 was identified in the subfraction EDG with retention time of 51.17 minutes. The mass spectrum (EI-MS) showed a molecular peak at m/z 426.00 and a base peak at m/z 189.10. Gammacer-16-en-3β-ol, ***5***: ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 3.20 (dd, J=5.3 and 11.1 Hz, H-3), 5.49 (dd, J=3.0 and 5.1 Hz, H-16), 0.99 (s, H-23), 0.77 (s, H-24), 0.84 (s, H-25), 0.94 (s, H-26), 0.98 (s, H-27), 1.04 (s, H-28), 1.06 (s, H-29), 1.10 (s, H-30); ^{13}C-NMR ($CDCl_3$, 150 MHz): Results are shown in Table 1; MS (m/z):426 (510.54), 411 (25.08), 393 (3.95), 218 (28.47), 204 (75.79), 203 (24.69), 189 (100.00), 177 (77.28), 150 (16.18), 135 (41.21). The presence of a Δ^{16}-double bond was corroborated by signals at δ 116.9 and 145.2 in the ^{13}C-NMR spectrum, assigned to C-16 and C-17, respectively, of gammacer-16-en-type skeletons (Shiojima *et al.,* 1992; Shiojima *et al.,* 1989).

Plant extracts containing pentacyclic triterpenes and their isolated compounds had been studied for their strong cytotoxic properties. Previously results suggested this kind of metabolites as potentially cytotoxic toward in neoplastic cells with promising results in the treatment of melanoma, glioma, laryngeal cancer, ovarian carcinoma, colon adenocarcinoma, pancreatic carcinoma, cervical carcinoma, epithelial carcinoma, prostate cancer, , hepatic cancer, breast cancer, colorectal cancer, colon cancer, gastric cancer, lung cancer, leukemia and neuroma (Chudzik *et al.,* 2015; Drag *et al.,* 2009; Gnoatto *et al.,* 2008; Kaweetripob *et al.,* 2013; Vazquez *et al.,* 2012).

Additional studies can be conducted with the hexane extract of *E dysenterica* as a promising source of pentacyclic triterpenes of natural origin.

Table 1: NMR ^{13}C dates from the pentacyclic triterpenes compared with literature data.

C	EDD	Mahato and Kundu (1994) ^{13}C (δ)		EDE	Sagar et al. (2004) ^{13}C (δ)	EDF	Swain et al. (2010) ^{13}C (δ)	EDG	Shiojima et al. (1989) ^{13}C (δ)
		(*1*)	(*2*)		(*3*)		(*4*)		(*5*)
1	38.6	38.7	38.7	41.7	41.9	38.1	38.0	38.8	38.7
2	27.2; 27.3	27.2	27.3	28.4	28.5	27.2	27.1	27.4	27.4
3	79.0; 79.1	78.3	79.0	79.1	79.5	79.0	79.1	79.1	79.1
4	38.6; 38.8	38.7	38.8	37.2	37.3	38.6	38.8	38.9	38.9
5	55.2; 55.3	55.2	55.3	55.5	55.6	55.5	55.5	55.2	55.3
6	18.3; 18.4	18.3	18.5	19.0	18.8	18.4	18.8	18.4	18.7
7	32.7; 32.8	32.9	32.8	33.4	33.4	35.1	35.1	33.4	33.5
8	40.1; 38.8	40.0	38.8	40.1	40.4	39.0	39.0	41.3	41.3
9	47.7	47.7	47.7	48.0	48.1	48.8	48.7	49.3	50.5
10	36.9; 37.6	36.9	37.6	34.3	34.1	37.8	37.7	37.2	37.1
11	23.4; 23.5	23.3	23.6	27.3	27.0	17.5	17.5	21.4	21.4
12	124.4; 121.7	124.3	121.8	124.4	124.8	35.8	35.8	22.7	22.7
13	139.6; 145.2	139.3	145.1	139.6	140.0	37.7	37.7	46.8	46.5
14	42.1; 41.6	42.0	41.8	42.1	42.5	158.1	158.1	39.6	39.4
15	28.8; 26.2	28.7	26.2	23.7	23.8	116.9	116.9	33.3	33.4
16	26.6; 27.0	26.6	27.0	27.9	27.7	36.7	36.7	116.9	117.8
17	33.7; 32.5	33.7	32.5	39.6	39.2	37.6	37.6	145.2	147.7
18	59.1; 47.2	58.9	47.4	59.1	59.5	49.3	49.3	37.7	37.7
19	39.7; 46.9	39.6	46.9	40.0	40.1	41.3	41.3	41.6	41.5
20	39.7; 31.1	39.6	31.1	39.9	40.0	28.8	28.8	18.3	18.2
21	31.3; 34.8	31.2	34.8	31.9	31.7	33.8	33.7	41.7	41.8
22	41.5; 37.2	41.5	37.2	39.6	39.2	33.1	33.1	36.7	36.1
23	28.1; 28.2	28.1	28.2	28.4	28.5	28.0	28.0	28.1	28.1
24	15.6; 15.4	15.6	15.5	16.1	16.1	15.4	15.4	15.5	15.5
25	15.5; 16.7	15.6	16.6	16.0	16.0	15.5	15.4	16.1	16.2
26	16.8; 16.9	16.8	16.9	18.0	17.9	29.9	29.9	16.9	16.9
27	23.3; 26.0	23.3	26.0	29.2	29.1	25.9	25.9	17.5	17.6
28	28.0; 28.4	28.1	28.4	23.7	23.7	29.7	29.8	29.9	29.9
29	17.5; 33.3	17.4	33.3	21.4	21.8	33.4	33.3	20.9	20.6
30	21.1; 23.7	21.3	23.7	17.5	17.3	21.3	21.3	33.4	33.5

CONCLUSION

Five pentacyclic triterpenes were identified in the hexane extract of *E dysenterica*, α-amyrin, β-amyrin, neolupenol, gammacer-16-en-3β-ol, and taraxerol. The compounds from *E. dysenterica* are widespread in higher plants, and present several confirmed biological activities. However, as far we know, it is the first report about triterpenes from hexane extract of *E. dysenterica* leaves.

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