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Maytenus salicifolia: triterpenes isolated from stems and antioxidant property of extracts from aerial parts

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Abstract: Six pentacyclic triterpenes were isolated from hexane extract of stems of *Maytenus salicifolia* Reissek, Celastraceae: 30-hydroxyfriedelan-3-one (**1**), 3,16-dioxofriedelane (**2**), friedeline (**3**), lupeol (**4**), betuline (**5**) and lup-20(29)-en-3,30-diol (6). The structure each one was established on the basis of detailed ^{1}H and ^{13}C NMR spectral investigation and by comparison with the respective literature values. For compound **1**, the complete 2D NMR (HMBC, HMQC and NOESY) spectral data were herein reported for the first time. Compounds **1**, **2, 5** and **6** were isolated for the first time from this plant. Antioxidant activity is described for some extracts from species of the Celastraceae family, then, the extracts from aerial parts of *M. salicifolia* were evaluated in relation to antioxidant potential using the DPPH method. Compared to quecertin, the AcEt extract (EAF) from leaves, AcEt (EAPF) and MeOH (EMPF) from pulp fruit and AcEt (EAT) and MeOH (EMT) from stems showed significant antioxidant property.

Article

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Introduction

Many species of the Celastraceae family have been phytochemically studied due to its pharmacological activities. The studies demonstrated that the principles of biological interest are associated to flavonoids, sesquiterpenes, alkaloids and pentacyclic triterpenes (PCTT) (Bruning & Wagner, 1978; Oliveira et al., 2006). Among the PCTT isolated from species of Celastraceae, friedelane, oleanane, lupane, taraxerane, ursane, dammarane and baccharane series represent the main constituents (Nuñez et al., 2005). Some of these compounds present pharmacological effects, like antitumoral (González et al., 2000) and antispermatogenic (Vieira Filho et al., 2002) activities.

The *Maytenus* genus, the biggest of the Celastraceae family comprises about eighty species which are distributed all over Brazilian territory (Oliveira et al., 2006). Pharmacological activities are popularly attributed to species of *Maytenus* genus (Nozaki et al., 1986; Reyes et al., 2006), and the antinociceptive (Jorge et al., 2004) and antioxidant (Vellosa et al., 2006) activities were experimentally recognized for *Maytenus ilicifolia.*

In Minas Gerais, Brazil, *Maytenus salicifolia* Reissek, Celastraceae, is commonly known as 'cafezinho' and its leaves have been traditionally used for the treatment of stomach ulcers. The tea (decoct) obtained from the fresh leaves is topically used to alleviate itches and symptoms of skin allergies (Valladão et al., 2009).

This work involves the phytochemical study of stems and due to the antioxidant activity showed by other species of *Maytenus* (Vellosa et al., 2006; Vicentino & Menezes, 2007), the antioxidant property of extracts from aerial parts of *M. salicifolia* was investigated The following PCTT were identified: 30-hydroxyfriedelan-3-one (**1**), 3,16-dioxofriedelane (**2**), friedeline (**3**), lupeol (**4**), betuline (**5**) and lup-20(29)-en-3,30-diol (**6**). The structural elucidation was carried out by 1D/2D (HSQC, HMBC and NOESY) NMR spectral data and comparison of the chemical shift assignments with data reported in the literature (Mahato & Kundu, 1994). The extracts with methanol (EMT), ethyl acetate (EAT) from stems, ethyl acetate (EAF) from leaves, and methanol (EMPF) and ethyl acetate (EAPF) from pulp fruit showed significant antioxidant property.

Material and Methods

Melting points were determined using a Mettler FP 80 HT apparatus. NMR spectra (1D and 2D) were obtained on a Bruker AVANCE DRX 400 or on Bruker DPX 200 spectrometers, using $CDCl_3$ as solvent and TMS as internal standard. IR spectra were recorded on a FITR-Perkin-Elmer, Spectrum One SN 74759 spectrophotometer. Column chromatography (CC) was carried out using silica gel 60 (Merck). TLC was performed using pre-coated silica gel plates, and the detection was obtained by spraying with solution (1:1) of vanillin (ethanol 1% solution w/v) in perchloric acid (3% aqueous solution v/v).

Maytenus salicifolia Reissek, Celastraceae, was collected at 'Serra de Ouro Branco', Ouro Branco, Minas Gerais-MG, Brazil, and a voucher (Nº. OUPR-18094) deposited at the *Herbarium* José Badini, UFOP-MG, Brazil. Leaves, stems and fruits were dried at room temperature (r.t.), fragmented in a mill and submitted to extraction with different solvents.

The *n*-hexane (EHF), ethyl acetate (EAF), *n*-butanol (EBF) and methanol (EMF) extracts from leaves, EtAc (EAPF) and MeOH (EMPF) extracts from pulp fruit and hexane (EHT), EtAc (EAT) and MeOH (EMT) extracts from stems were submitted to antioxidant evaluation using 2,2-diphenyl-1-picrilhydrazyl (DPPH) method. Each extract was dissolved in methanol at concentrations of 10.0, 20.0, 30.0, 40.0, 50.0 and 100.0 µg/mL into the test tubes and methanol solution of DPPH (Sigma) (150 µg/mL) was added in each one and kept at 25 a 30 ºC. Methanol solution of DPPH was used as blank, methanol as a broker of the baseline and quecertin as positive control. The absorbance was measured at 517 nm on a α-Helios Spectrophotometer, Thermo Electron Corporation, USA. Controls were used in the measure of all concentrations of the extracts tested for detection of possible interferences in the absorbance. All assays were performed in triplicate and expressed as the mean. The antioxidant percentage was established in according to formula suggested by Singh et al. (2002). The results (Figure 1) were compared to quecertin which is known by its free radical scavenging property.

The fragmented stem (2525.86 g) was subjected to hexane extraction at r. t. During the hexane withdrawing, a white solid material precipitated and was filtered, dried (0.470 g) and submitted to silica gel 60 (20.0 g) CC, eluted with hexane (100%), hexane-chloroform (8:2), *idem* (1:1), chloroform (100%), chloroform-ethyl acetate (8:2), *idem* (1:1), ethyl acetate (100%) and methanol (100%), furnishing eighteen fractions of 10.0 mL. After solvent evaporation, fraction 3 gave a white solid (13.0 mg), which was identified by NMR spectrometry as triterpene **3**. The fraction 7 (293.0 mg) was fractionated by CC eluted in same conditions, resulting in 24 fractions of 5.0 mL. From this process, fractions 9 to 20 grouped was identified as triterpene **2** (227.0 mg), and fraction 22 furnished compound **1** (27.0 mg). The complete ^1H and ^{13}C NMR spectral data of **1** are listed for the first time in Table 1.

The dried residue (14.652 g) obtained from hexane extraction was subjected to silica gel 60 (600.0 g) CC, eluted with hexane (100%), hexane-chloroform (9:1), idem (8:2), *idem* (1:1), chloroform (100%), chloroform-ethyl acetate (9:1), (8:2), *idem* (1:1), ethyl acetate (100%) and methanol (100%), resulting in 52 fractions of 200.0 mL each. Through the similar profile observed in TLC, thirteen groups of fractions were separated. Group 9 furnished compound **4** (25.0 mg), and group 10 gave **5** (20.0 mg). Group 11 (2.483 g) was submitted to silica gel 60 (100.0 g) CC, which was eluted with the same eluent system cited above. By this process fourteen fractions were obtained, and among them, fraction 8 was identified as been triterpene **6** (32.0 mg).

Results and discussion

Through the NMR data were identified two series of PCTT isolated from stems *Maytenus salicifolia* Reissek, Celastraceae: compounds **1, 2** and **3** of the friedelane series, and **4, 5** and **6** of the lupane series. These compounds gave a Liebermann-Buchard test positive for triterpenes (Valladão et al., 2009). The structural elucidation of the PCTT **1** to **6** was based on its ^1H and ^{13}C NMR spectral data, including for compound **1**, the 2D (HSQC, HMBC and NOESY) data.

Triterpene **1** (27.0 mg) was firstly isolated from *Catha cassinoides* (Betancor et al., 1980), and only 1D NMR spectral data were reported for this compound. By the analysis of IR spectrum of **1** were observed absorption bands at 3533 cm^{-1}, characteristic of OH group, at 1715 cm^{-1} associated to C=O group, and at 1059 cm^{-1}, attributed to saturated primary alcohol (Barbosa, 2007). Through ^{13}C NMR spectrum of 1 was possible to identify the presence of 7 CH_3, 12 CH_2, 4 CH and 7 quaternary carbons (Table 1). The characteristic downfield signal at δC 71.90 was attributed to carbon attached to hydroxyl group. The complete NMR chemical shift assignments were performed through HMBC, HSQC, and NOESY. The HMBC contours map showed correlations of the signal at δC 213.30 (C=O) with δH 2.37 (H-2), δH 2.23 (q, H-4) and δH 0.87 (d, H-23). Consequently, the signal at δC 213.30 was attributed to carbon C-3. Through comparison of the literature data (Mahato & Kundu, 1994) with the correlations observed in HSQC spectra, between δC 71.90 with δHa 3.35 and 3.43, δC 59.44 with δH 1.54, these carbon signals were respectively attributed to C-30 and C-10. The correlations of the signal at δC 6.83 with δH 0.87, and δC 14.65 with δH 0.73, allowed to attribute the chemical shift of C-23

and C-24. In HMBC spectra were observed correlations between the signal of carbinolic hydrogen (δHa 3.35 and δHb 3.43) with two signals: one associated to non-hydrogenated carbon (δC 33.36; C-20) and other correspondent to methyl group (δC 28.93; C-29). These correlations sequence confirm the position of the hydroxyl group at C-30. In the NOESY map, was observed that the signal of hydrogen H-30a (δH 3.35) correlates with the signal of H-18 (δH 1.51) confirming the position of hydroxyl in C-30. NOESY correlations of H-24 (δH 0.73) with H-23 (δH 0.87) and H-28 (δH 1.15) with H-30b (δH 3.43) also were observed. The HMBC, HSQC and NOESY data confirm the relative stereochemistry of 30-hidroxyfriedelan-3-one (**1**). This is the first time that 2D NMR spectral data were attributed for compound **1**.

Triterpene **2** (227.0 mg) was isolated as a white solid. The IR spectrum presented absorption bands correspondent to group CH (2970 cm^{-1} and 2849 cm^{-1}) and C=O (1715 cm^{-1} and 1687 cm^{-1}) (Barbosa, 2007). The ^{13}C NMR and DEPT-135 spectra of **2** showed signals of 8 CH_3, 10 CH_2, 4 CH and eight quaternary carbons. There were observed signals at δC 218.97 and at δC 213.67, attributed to carbonyl at C-3 and C-16. Compound 2 (3,16-dioxofriedelane) was for the first time isolated from *Maytenus diversifolia* (Nozaki et al., 1986).

The ^{13}C NMR spectrum of triterpene 3 (13.0 mg) showed signal at δH 214.59 (C=O) and signals at δH 6.73 and δH 14.56, attributed to methyl groups, which with the absence of double bonds signals suggested a friedelane structure (Mahato & Kundu, 1994). Triterpene **3** was identified as friedelin,

Table 1. 1D and 2D NMR* spectral data of **1** (30-hydroxyfriedelan-3-one).

Nº	δC	Type	δH	HMBC	NOESY
1	22.27	CH_2	1.97 (m)	C-10	CH_2(2)ax., Me (24)
2	41.50	CH_2	2.37 (m)	C-10, C-3	H-C (10), Me (23)eq
3	213.30	C			
4	58.19	CH	2.23; (q) J=6.8	C-5, C-23, C-24	Me (23), CH_2 (7)eq
5	42.15	C			
6	41.24	CH_2	1.77 (m)	C-8	
7	18.23	CH_2	1.41 (m)	C-8	CH_2 (15) eq, Me (24)
8	53.00	CH	1.40 (m)	C-15	
9	37.42	C			
10	59.44	CH	1.54 (m)	C-1	CH_2(2)ax
11	35.55	CH_2	1.58 (m)		
12	29.71	CH_2	1.51 (m)	C-11	
13	39.77	C			
14	38.38	C			
15	32.12	CH_2	1.49 (m)	C-13	CH_2 (7)eq
16	29.71	CH_2	1.22 (m)		
17	29.96	C			
18	42.69	CH_2	1.51 (m)		CH_2 (12)ax
19	30.50	CH_2	1.43 (m)		
20	33.36	C			
21	28.15	CH_2	1.19 (m)	C-30	
22	39.77	CH	1.66 (m)		
23	6.83	CH_3	0.87 (d); J=6.8	C-3, C-4, C-5	CH_2 (1)ax., CH_2 (6)eq
24	14.65	CH_3	0.73 (s)	C-4, C-5, C-6, C-10	Me(23)
25	18.01	CH_3	0.90 (s)	C-8, C-9, C-10, C-11	
26	18.59	CH_3	1.07 (s)	C-8, C-13, C-14, C-15	
27	19.95	CH_3	0.99 (s)	C-12, C-13, C-14, C-18	CH (8)ax., CH_2 (19)eq., CH_2 (15)ax
28	32.12	CH_3	1.15 (s)	C-16, C-17, C-18, C-22	
29	28.93	CH_3	1.01 (s)	C-19, C-20, C-21, C-30	
30	71.90	CH_2	3.35(d, Ha); 3.43(d, Hb) J_a=J_b=10.4	C-29, C-20	(H_a=H_b) Me(28), CH(18)

*^{1}H (400MHz) and ^{13}C (100 MHz), $CDCl_3$ solution; δ in ppm, J in Hz.

which was previously isolated from hexane extract of the leaves of *M. salicifolia* (Miranda et al., 2006). Friedelin and 3β-friedelinol has been used in quality control of species of the genera *Maytenus*, particularly of *Maytenus ilicifolia* (Valladão et al., 2009).

The ^{13}C NMR spectrum of triterpene **4** (25.0 mg) showed signal at δC 109.68 and δC 150.47, attributed to olefinic carbon, and at δC 78.97 associated to carbinolic carbon (C-3). By the comparison of carbon signals with literature data (Mahato & Kundu, 1994), compound 4 was identified as lupeol (Miranda et al., 2006). This triterpene is found in fig, mango and other fruits, and has paying attention due to its antioxidant activity, induction of apoptosis and antiproliferative properties (Nigam et al., 2009).

Triterpene **5** (20.0 mg) was isolated as a white solid. The IR spectrum showed absorption bands of CH (2921 cm^{-1} and 2851 cm^{-1}) and OH (3404 cm^{-1}) (Barbosa, 2007). The ^{13}C NMR and DEPT-135 data of **5** showed signals of 6 CH_3, 12 CH_2, 6 CH and six quaternary carbons. The signals at δC 78.77 and δC 60.36 were associated to hydroxylated carbons C-3 and C-28 (Mahato & Kundu, 1994). Compound 5 was identified as betuline. This triterpene showed inhibitory effects on production of NO and prostaglandin E2 in mouse macrophages (Reyes et al., 2006).

Triterpene **6** (32.5 mg) was isolated as a white solid. Its IR spectrum showed absorption bands of OH (3277 cm^{-1}) and CH (2936 cm^{-1} and 2870 cm^{-1}) (Barbosa, 2007). The ^{13}C NMR and DEPT-135 spectra of **6** indicate 5 CH_3, 12 CH_2, 6 CH and six quaternary carbons. The ^{13}C NMR data of 6 were in agreement with the data reported for lup-20(29)-en-3β,30-diol, firstly isolated from *Maerua oblongifolia*, Capparaceae (Abdel-Mogib, 1999).

The (DPPH) stable free radical photocolorimetric method is frequently used to evaluate the antioxidant property of different extracts from plants (Falcão et al., 2006; Vicentino & Menezes, 2007; Nunes et al., 2008). In relation to the antioxidant property of *M. salicifolia*, the extracts EHF, EBF, EMF and EHT were considered

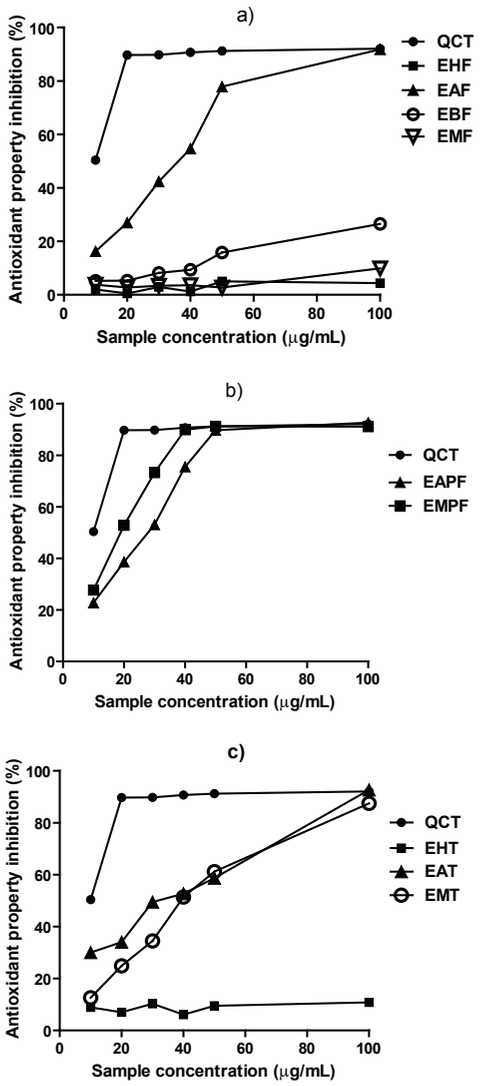
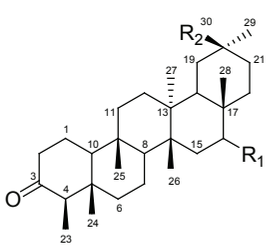
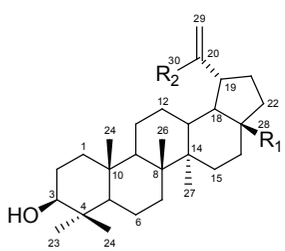


Figure 1. Antioxidant property of *Maytenus salicifolia* Reissek, Celastraceae, extracts, in comparison with quecertin (QCT). a) hexane (EHF), ethyl-acetate (EAF), butanol (EBF) and methanol (EMF) extracts from leaves, b) ethyl-acetate (EAPF) and methanol (EMPF) extracts from fruit, and c) hexane (EHT), ethyl-acetate (EAT) and methanol (EMT) extracts from stem.



Friedelane	R_1	R_2
1	H	CH_2OH
2	=O	CH_3
3	H	CH_3



Lupane	R_1	R_2
4	CH_3	CH_3
5	CH_2OH	CH_3
6	CH_3	CH_2OH

inactive, but the extracts EMT and EAF showed significant activity at concentrations above 40.0 µg/mL, EAPF and EAT>30.0 µg/mL and EMPF>20.0 µg/mL (Figure 1). Since that was not found antioxidant property in EHT, the triterpenes isolated from this extract were not tested.

In this paper, the antioxidant property of extracts from the leaves, fruits and stems of *M. salicifolia* and the isolation of 30-hydroxyfriedelan-3-one (**1**), 3,16-dioxofriedelane (**2**) betuline (**5**) and lup-20(29)-en-3,30-diol (**6**) are herein reported for the first time.

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