SUPPLEMENTARY MATERIAL

Chemical Constituents of *Eugenia catharinae* and their antioxidant activity

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Abstract

Nine compounds were isolated from the leaves of *Eugenia catharinae*, namely monomethyl olivetol (**1**), β -sitosterol (**2**), stigmasterol (**3**), uvaol (**4**), erythrodiol (**5**), rotundic acid (**6**), quercetin (**7**), catechin (**8**) and myricitrin (**9**). The structures of 1-9 were established through analysis of their spectroscopic (^1H and ^{13}C NMR) and spectrometric (MS) data. Compounds **1** and **6** are reported the first time in the *Eugenia* genus. In addition these data were compared with those reported in the literature. The antioxidant activity of plant samples and compounds was measured using the DPPH radical scavenging assay. Flavonoids **7**, **8**, **9** and the ethanolic extract showed the best results, with IC_{50} values of 20.94 μM , 44.20 μM , 30.01 μM and 58.82 $\mu\text{g/mL}$, respectively.

Keywords: *Eugenia catharinae*; DPPH; rotundic acid; monomethyl olivetol

Experimental

Plant material

The leaves of *Eugenia catharinae* were collected in Florianópolis, Santa Catarina State, Brazil, in July 2010, from a healthy and agrotoxic-free plant. Botanical identification was performed by Dr. Daniel de Barcellos Falkenberg. A voucher specimen (FLOR 27820) has been deposited in the Herbarium FLOR, at the Universidade Federal de Santa Catarina, Santa Catarina, Brazil.

General experimental procedures

UV analyses were recorded on a Perkin-Elmer Lambda2S UV/VIS spectrophotometer. Infrared (IR) spectra were recorded on ABB FTIR – FTLA2000 spectrometer with KBr and expressed in cm^{-1} . Nuclear magnetic resonance spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C on a VARIAN NMR AS 400 spectrometer taking TMS as internal standard. Column chromatography was performed on silica gel (60-120 mesh, Carvalhaes, Macherey-Nagel, Germany). Thin layer chromatography (TLC) was performed on a pre-coated silica gel type-60 plate (Macherey-Nagel).

Extraction and Isolation

Dried and pulverized leaves of *E. catharinae* (475.2 g) were extracted at room temperature (ca. 25°C) with 96% EtOH to give 73.9 g of crude extract. The crude extract was suspended in an EtOH-H₂O (1:1) solution and stored in a refrigerator for one day. After this procedure, the extract was filtered and an insoluble material was obtained (26.8 g). The filtrate was then partitioned into hexane, EtOAc and BuOH. The solvents were evaporated to give hexane (9.10 g), EtOAc (22.3 g), BuOH (7.05 g) and aqueous (8.55 g) fractions.

The insoluble material (26.0 g) was chromatographed over silica gel using hexane and EtOAc with increasing polarity. Sixty-nine fractions (A1-A69) were collected, analyzed by TLC and then grouped according to their chromatographic profiles. From fractions A13-A20, monomethyl olivetol (**1**) was obtained as an orange oil (627.6 mg). Chromatography of the A23-A30 fractions (87.6 mg) using a gradient system of hexane/EtOAc (10:0 to 0:10) afforded twenty-one subfractions (B1-B21). Two of these fractions (B5-B6), after grouping, were purified by successive recrystallization with MeOH, providing 7.62 mg of a mixture of steroids, β -sitosterol (**2**) and stigmasterol (**3**).

A sample (7.50 g) of the hexane fraction was chromatographed over silica gel, eluting with hexane while gradually increasing the polarity with EtOAc and EtOH. Twenty-three

fractions (C1-C23) were collected, analyzed by TLC and then grouped according to their chromatographic profiles. Fractions C4-C9, after grouping, were purified by successive recrystallization with EtOAc and MeOH providing 53.0 mg of a mixture of triterpenes, uvaol (**4**) and erythrodiol (**5**).

The EtOAc fraction (10.0 g) was chromatographed over silica gel, eluting with hexane while gradually increasing the polarity with EtOAc and EtOH. Twenty-nine fractions (D1-D29) were collected, analyzed by TLC and then grouped according to their chromatographic profiles. Flash column chromatography of the D8-D9 fraction (170 mg) using hexane:EtOAc (20:80) as an isocratic eluting mixture afforded sixty-three subfractions (E1-E63). The subfractions E4-E6 and E11-E48 after successive recrystallization in methanol yielded 8.10 mg of rotundic acid (**6**) and 33.2 mg of quercetin (**7**), respectively. The fractions D12 and D17 were purified by successive recrystallization with CHCl_3 , providing 96.8 mg of catechin (**8**) and 88.3 mg of myricitrin (**9**), respectively.

Antioxidant activity

Antioxidant activity of the isolated compounds was evaluated using assays to determine the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) scavenging activity. For this assay 1 mL of the ethanol sample (5-100 $\mu\text{g/mL}$) was added to 2 mL of a solution of DPPH radicals in methanol (0.004%). The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. In order to subtract the absorbance promoted by staining of sample an ethanol (2.0 mL) and isolated compound (1.0 mL) solution was used as the blank ($\text{Abs}_{\text{blank}}$). A DPPH (2.0 mL) and ethanol (1.0 mL) solution was used as the control ($\text{Abs}_{\text{control}}$) which is regarded as 100% of DPPH. The absorbance ($\text{Abs}_{\text{sample}}$) of the resulting solution was measured at 517 nm and converted into percentage of antioxidant activity (AA) using the following formula: $\text{AA}\% = 100 - \{[(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100] / \text{Abs}_{\text{control}}\}$. Ascorbic acid was used as standard. The radical scavenger activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC_{50}). The IC_{50} value for each sample was determined graphically by plotting the percentage disappearance of DPPH as a function of the sample concentration.

Figure S1. ¹H-NMR (400 MHz, CDCl₃) Spectrum of Compound **1**

Figure S2.APT (100 MHz, CDCl₃) Spectrum of Compound **1**

Figure S3. ^1H -NMR (400 MHz, CDCl_3) Spectrum of mixture of steroids **M1**

Figure S4. ¹³C-NMR (100 MHz, CDCl₃) Spectrum of mixture of steroids **M1**

Figure S5. ^1H -NMR (400 MHz, CDCl_3) Spectrum of mixture of triterpenes **M2**

Sample:
File: exp
Pulse Sequence: s2pu1
Solvent: CDCl3
Temp. 26.0 C / 298.1 K
Operator: Rotina
Mercury-400BB "RMN.qmc.UFSC.br"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.300 sec
Width 24154.5 Hz
4093 repetitions
OBSERVE C13, 100.5892887 MHz
DECOUPLE H1, 400.0366320 MHz
Low power 10 dB atten.
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
F1 size 65536
Total time 2 hr, 39 min, 33 sec

Figure S6. ^{13}C -NMR (100 MHz, CDCl_3) Spectrum of mixture of triterpenes **M2**

Figure S7. ^1H -NMR (200 MHz, Py- d_5) Spectrum of Compound 6

Figure S8. ^{13}C -NMR (50 MHz, Py-d5) Spectrum of Compound **6**

Figure S9. ^1H -NMR (400 MHz, CD_3OD) Spectrum of Compound 7

Figure S11. ¹³C-NMR (100 MHz, CD₃OD) Spectrum of Compound 8

Figure S12. ¹H-NMR (400 MHz, CD₃OD) Spectrum of Compound 9

Figure S13. ¹³C-NMR (100 MHz, CD₃OD) Spectrum of Compound **9**