Polyphenolic profile by FIA/ESI/IT/MS^n and antioxidant capacity of the ethanolic extract from the barks of *Maytenus cajalbanica* (Borhidi & O. Muñiz) Borhidi & O. Muñiz.

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Abstract

Medium and high polarity extracts from *Maytenus* species are known to contain polyphenolic compounds such as proanthocyanidins. The high polarity and structural complexity of these compounds make very difficult their isolation even by modern chromatographic techniques. *Maytenus cajalbanica* (Borhidi & O. Muñiz) Borhidi & O. Muñiz is endemic from Cuba. So far, there are reports neither of phytochemical work nor of biological evaluation of extracts from this subspecies. The goal of this work is to determine the polyphenolic profile and the antioxidant capacity of the ethanolic extract from the barks of *Maytenus cajalbanica*. FIA/ESI/IT/MS^n analysis allowed the identification of 5 flavan-3-ol monomers, 33 proanthocyanidins, 2 free flavonoids and their respective glycosides as major compounds of the ethanolic extract, which showed a strong radical scavenging capacity and a significant ferric reduction power. FIA/ESI/IT/MS^n technique led the rapid, effective and sensitive determination of the polyphenolic profile of *Maytenus cajalbanica* without previous separation.

Keywords: *Maytenus*, Celastraceae, proanthocyanidins**,** FIA/ESI/IT/MS^n, antioxidant activity.

Experimental

Plant material

Maytenus cajalbanica barks were collected from La Palma, Sierra de Cajálbana, Pinar del Río, Cuba in December 2012. Plant material was identified by MSc. Ramona Oviedo from the Botanic Garden of Cuba. A voucher specimen (No. HAC 43117) was deposited in the herbarium Institute of Systematic and Ecology of Cuba.

Maytenus cajalbanica extracts

Fresh material was oven-dried at 40 ^{0}C and ground to powder. 330 g were defatted with diethyl ether/ petroleum ether then extracted with ethanol (70%). The solution was filtered using a vacuum pump and the solvent was evaporated in a rotary evaporator to obtain the ethanolic extract (yielding 23 g).

Sample preparation for FIA/ESI/IT/MS^n analysis

For mass spectrometric analysis, 2 mL of a MeOH/H_2O 8:2 (v/v) solution of the ethanolic extract (1 mg/ ml) was submitted to the solid-phase extraction using RP18 cartridge, then eluted with H_2O/MeOH 8:2 (v/v). After drying, 1 mg was dissolved in 1 mL of the solvent mixture MeOH/H_2O 8:2 (v/v) (solution A) and an aliquot (10 µL) was diluted with MeOH/H_2O 8:2 (v/v) up to a final volume of 1 mL, and then filtered through a 0.22 µm membrane of a nylon filter. The solution (150 µL) was diluted with MeOH/H_2O 8:2 (v/v) up to a final concentration of 1 ppm and then introduced by direct flow injection at 5 µL /min into the ESI source using a syringe pump.

FIA/ESI/IT/MS^n analysis

FIA/ESI/MS^n analyses were performed using a Thermo Finnigan LCQ Deca ion trap mass spectrometer (San Jose, CA, USA). Analyses in negative and positive ionization modes were performed. The data were acquired in the full scan (range of *m/z* 50–2000) and tandem mass scanning modes. For MS^n analyses, collision energies chosen for each fragmentation was 35%. The optimized instrumental parameters were capillary temperature 300^{0}C; capillary voltage13V; spray voltage 5kV; sheath gas flow rate 35 (nitrogen, arbitrary units); auxiliary gas flow rate 10 (arbitrary units).

DPPH$^{\bullet}$ radical scavenging assay

The free radicals scavenging capacity was determined as previously described (Brand-Willians W 1995). In the assay, an ethanolic solution of 130 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$, Sigma, USA) was mixed with plant extract (2-1000 µg/mL). Ascorbic acid (Sigma) was employed as standard. The reaction mixtures were incubated in the dark at room temperature for 30 min and the absorbance was measured at 515 nm. The inhibition percent of DPPH$^{\bullet}$ radical was calculated by the following equation: Inhibition (%) = (D.O. control – D.O. sample)/D.O. control) × 100. The concentration of the dried extract, required to scavenge 50% of DPPH$^{\bullet}$ (IC_{50}), was also determined.

FRAP assays

The reducing capacity was measured according to the method of Benzie and Strain (Benzie IF 1996). Briefly, acetate buffer (300 mM, pH=3.6), TPTZ (2,4,6-tripyridyl-s-triazine; Sigma) 10 mM in 40 mM HCl and $FeCl_3$ x $6H_2O$ (20 mM) were mixed in the ratio of 10:1:1 to obtain the working FRAP reagent. The plant extract (20 µL; 0.1 mg/mL) was mixed with 900 µL of FRAP reagent. The mixture was incubated at room temperature for 4 min and absorbance was measured at 593 nm. A solution of Ascorbic acid (100 µM) was used as standard.

Table S1: Antioxidant capacity of the ethanolic extract from the barks of *Maytenus cajalbanica* by DPPH and FRAP methods.

	DPPH **IC50 ± S.D (µg/mL)** **(% inhibition ± S.D)**	**FRAP** **(O.D. 593 nm ± S.D)**
Ethanolic extract	11.37 ± 2.25 (86.29 ± 0.86%)[a]	0.876 ± 0.011[a]
Ascorbic acid	20.35 ± 3.19 (61.08 ± 3.19)[b]	0.891 ± 0.019 [a]

The values represent the mean ± standard error of mean of experimental data. Different letters represent statistical differences between the same set (t-student test, $p<0.05$).

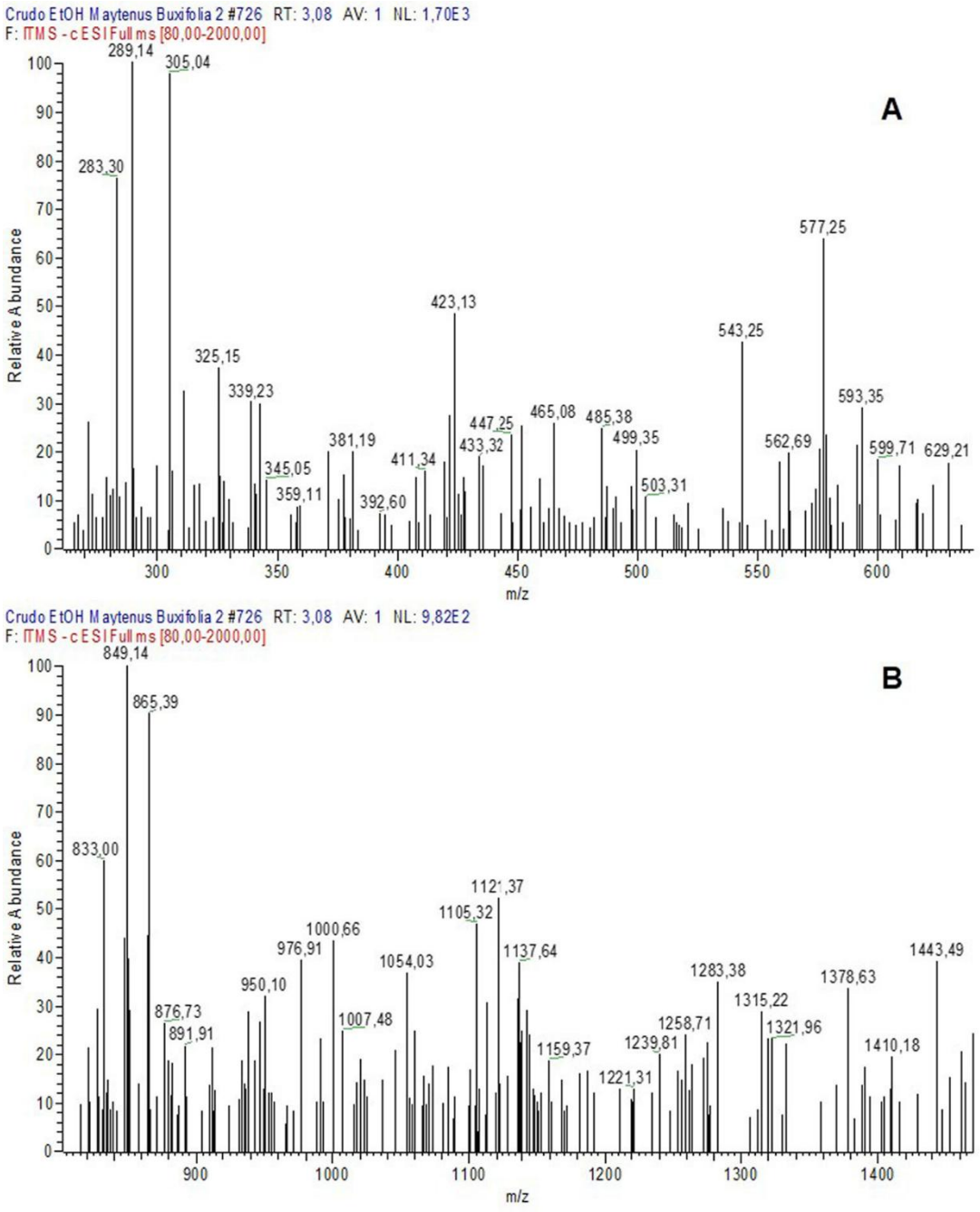

Figure S1. Full scan mass spectrum of ethanolic extract. A: *m/z* range 260-630. B: *m/z* range 800-1460.