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Traditional Use, Chemical Constituents, and Pharmacological Activity of *Maytenus elaeodendroides* Stem Bark

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Abstract: Plants belonging to the genus *Maytenus* are members of the *Celastraceae* family. They have been widely used by different peoples as treatment for curing many diseases. The aim of this study was to explore the anti-inflammatory and antioxidant properties of *Maytenus elaeodendroides* stem bark extracts, an endemic Cuban plant. The antioxidant activity of four extracts (EtOH, EtOAc, *n*-BuOH, and diethyl ether/petroleum ether 1:1) was determined using DPPH and FRAP methods. Meanwhile, anti-inflammatory effects by the edema method were induced by croton oil in the mouse ear. The investigated extracts showed radical reduction capacity and prevented ear inflammation at doses of 4 mg/ear. In addition, FIA/ESI/IT/MS^n was used to determine the qualitative chemical composition of the EtOAc extract and allowed the identification of five flavan-3-ol monomers, four dimers, and other proanthocyanidin oligomers. From this extract three flavan-3-ol compounds (elaeocyanidin and 4′-*O*-methylgallocatechin), one of them new (2′-hydroxy-4′-methoxy-epigallocatechin), and a proanthocyanidin dimer (afzelechin-(4β→8)-4′-*O*-methylepigallocatechin) were isolated and identified by the chromatographic method and spectroscopic techniques, mainly ESI-MS and NMR spectroscopic methods.

Keywords: *Maytenus elaeodendroides*; flavan-3-ol; 2′-hydroxy-4′-methoxy-epigallocatechin; proanthocyanidins; FIA/ESI/IT/MS^n; antioxidant and anti-inflammatory activity

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1. Introduction

Medicinal plants play a fundamental role in the health systems of many countries. Nowadays, medicinal plants still represent a precious source of inspiration for the research and development of new drugs [1,2]. The family Celastraceae includes 106 genera with approximately 1300 species [3] and is widespread in tropical and sub-tropical regions around the world [4]. The family Celastraceae are widely used in traditional medicine [5] (González et al., 2000). Particularly, *Maytenus* species are widely used in folk medicine and several medicinal uses are associated with it, such as antitumor, anti-asthmatic, treatment for stomach problems, antioxidant, analgesics, anti-inflammatories, and antimicrobials [6–10]. Over the years, the numerous biological activities attributed to *Maytenus* species has prompted

several phytochemical studies and many compounds' classes were characterized and isolated, including flavonoids, pentacyclic triterpenes, alkaloids, and condensed tannins [3]. Our previous studies on the *M. aquifolium* Martius e *M. ilicifolia* Mart. ex Reiss allowed the isolation and structural characterization of a series of new flavonoid tetraglycosides [11,12]. Recently, other authors have reported the effectiveness of *Maytenus ilicifolia* extracts in preventing low-density lipoprotein (LDL) oxidation, a significant factor in reducing the risk of cardiovascular disease [13]. Medium and high polarity extracts from *Maytenus* species are known to contain polyphenolic compounds such as flavan-3-ol and proanthocyanidins [13,14]. The isolation of proanthocyanidins is very difficult due to their great structural complexity, polarity, and high molecular weight. Therefore, tandem mass spectrometry in the flow injection analysis mode (FIA/ESI/IT/MS^n) technique is a very useful tool for the identification of these compounds. FIA/ESI/IT/MS^n was used to determine the polyphenolic profile of the ethanolic extract of *M. cajalbanica* barks, identifying 5 flavan-3-ol monomers, 33 proanthocyanidins, 2 free flavonoids, and their respective glycosides as major compounds [15]. *M. elaeodendroides* has been used for many years in Cuban traditional medicine for the treatment of inflammatory processes [16]. In previous works, Spengler et al. isolated, from the less polar extract of *M. elaeodendroides* bark, triperpenes belonging to the family of lupeol and friedelane [17,18]. In addition, Fernández et al. reported the isolation of four triperpenes of lupeol for the first time in the species *M. elaeodendroides* [19]. There are reports in the literature on the anti-inflammatory activity of lupane triterpenes, isolated in the studies cited above [17,19,20]. Caruso et al. [21] explored the *Maytenus octogona* potential as an antioxidant and anti-inflammatory agent through two key methodologies: cyclic voltammetry and computational docking. They highlighted the potential therapeutic applications of *M. octogona* in managing oxidative stress and inflammation, suggesting that it may be valuable in treating conditions related to these processes. New flavonoid (−)-4′-*O*-methylepicatechin 5-*O*-β-D-glucopyranoside, along with four known triterpenes and a flavonoid were isolated recently from the ethyl acetate extract of *Maytenus quadrangulata* leaves. The compounds were evaluated against the bacteria *Staphylococcus aureus* and *Klebsiella pneumoniae*, but neither exhibited activity, even at the highest concentration tested [22]. Recently, inhibitory activity of Mayaro virus replication and infectivity exerted by *Maytenus quadrangulata* extracts has been reported. This finding is relevant given the lack of specific antiviral treatments for many viral infections [23]. The study showed the contribution of catechin to the overall antiviral effect of the extract, perhaps interfering with the viral life cycle or enhancing the host immune response. To gain knowledge of folk uses and traditional plants growing in Cuba, the purpose of this study was to characterize the phytochemical profile of four extracts obtained from stem bark of *M. elaeodendroides* and to evaluate its bioactive properties, as well as its antioxidant and anti-inflammatory activities.

2. Materials and Methods

2.1. General

Analytical-grade petroleum ether, *n*-hexane, ethyl acetate (EtOAc), *n*-butanol (n-BuOH), methanol (MeOH), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), and ethanol (EtOH) were used in this work. For FIA-ESI-IT-MS^n, HPLC-grade methanol was purchased from J.T. Baker (Phillipsburg, NJ, USA). HPLC-grade water was prepared using a Milli-Q purification system (Millipore, Bedford, MA, USA). The RP18 cartridge was a Phenomenex Strata C18-E, 55 µm, 70 Å, 500 mg·3 mL^{-1}. The filter membrane (0.22 µm) was nylon. Sephadex LH-20 and silica gel 0.06-0.2 mm (70-230 Mesh, Merck, Rahway, NJ, USA) were used for column chromatography and TLC was performed on 0.2 mm thick Kiesegel 60 F254 layers (Merck, NJ, USA).

2.2. Plant Material

The stem bark of *Maytenus elaeodendroides* was collected in La Coca dam in December 2011 and identified by Dr. Pedro Herrera from Instituto de Ecología y Sistemática (Havana,

Cuba), where a voucher specimen was deposited with the number HAC41417. The plant material was oven-dried at 40 °C and ground to a fine powder, yielding 990 g.

2.3. Extraction and Isolation

The powdered oven-dried plant samples (250 g) were defatted with diethyl ether/petroleum ether 1:1 (ME-4 extract) then extracted with EtOH. The EtOH extract (ME-1) was concentrated by reduced pressure to yield an oil, further dissolved in a hydro alcoholic solution (30%) and extracted with EtOAc and n-BuOH in a separating funnel. The resulting extracts were concentrated under reduced pressure in order to obtain the final EtOAc extract (ME-2) (21.3 g) and n-BuOH extract (ME-3) (14.4 g). The EtOAc (3 g) extracts were further fractionated with an n-hexane/$CHCl_3$/MeOH (1:2:3) mixture through Sephadex LH-20 packed column to give ten fractions (A-J). Fractions E and F were further fractionated by column chromatography on silica gel with n-hexane/$CHCl_3$/MeOH (1:1:0.4) to give 6 and 5 fractions. Fractions E2 and F3 were purified by preparative thin layer chromatography with n-hexane/$CHCl_3$/MeOH (1:1:0.4) mixture to give the compounds **1** (3 mg) and **3** (21 mg)**.** Compounds **2** (18 mg) and **4** (22 mg) were obtained by preparative thin layer chromatography from fractions E5 and F4 with CH_2Cl_2/EtOAc/MeOH (1:1:0.3).

2.4. Structure Elucidation Procedures

The structural elucidation was carried out through physical and spectroscopic data measurements, and by comparing the obtained data with previously published values. The melting points (m.p.) were determined on a Electrothermal 9100 apparatus. The optical rotations were determined with a Jasco p-1020 automatic polarimeter. FAB-MS data were measured by a JEOL JMS HX 110 spectrometer (Corporate benefits, Peabody, MA, USA), and the positive ions were detected by using glycerin as a matrix. ^{1}H- and ^{13}C-NMR spectra were obtained using a Varian Unity Inova 500 spectrometer (International Equipment Trading Ltd., Mundelein, IL, USA) in deuterated dimethylsulfoxide (DMSO-d_6), using TMS as an internal standard. Mass spectra were recorded on an LCQ Fleet mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Thermo Finnigan LCQ Deca mass spectrometer, both equipped with an ESI source and a direct injection device for the sample.

Compound **1**

Red solid; mp 160–161 °C (recrystallized acetone). $[\alpha]_D^{20^\circ} = -3.7$ (c = 0.0029 mol/L, CH_3OH); ESI-HRMS, *m/z* 342.1222 (100%) (positive mode) from the deuterated molecular ion peak; Anal. Calcd for $C_{16}H_{10}D_6O_8$: C 56.09%, H 6.30%, O 37.32%. Found: C 56.14%, H 6.47%, O 37.39%. NMR-^{1}H (DMSO-d6, ppm): 2.48 (1H, d, J = 3.5 Hz; 16 Hz); 2.68 (1H, d, J = 4.5 Hz, 16 Hz); 3.66 (3H, s); 4.1 (1H, d, J = 2.5 Hz); 4.68 (1H, s); 5.72 (1H, d, J = 2.0 Hz); 5.89 (1H, d, J = 2.0 Hz); 6.41 (1H, s). NMR-^{13}C (DMSO-d6, ppm): 77.8; 64.6; 28.0; 157.5; 95.0; 157.7; 93.9; 156.6; 100.0; 136.6; 105.95; 149.8; 134.4; 149.8; 105.95; 59.38.

2.5. Flow Injection Analysis (FIA-ESI-IT-MS^n)

A solution of EtOAc extract (MeOH/H_2O 8:2 *v*/*v*, 1 mg/mL) was submitted to the solid-phase extraction using an RP18 cartridge, eluted with MeOH/H_2O 8:2 (*v*/*v*). After drying, 1 mg was dissolved in 1 mL of MeOH/H_2O 8:2 (*v*/*v*) solution (solution A) and an aliquot was diluted with MeOH/H_2O 8:2 (*v*/*v*) to reach a final volume of 1 mL (1 µg/mL) and was filtered through a 0.22 µm nylon filter membrane. The final solution was introduced by direct flow injection at 5 µL/min into the ESI source using a syringe pump. Analyses were performed using a Thermo Finnigan LCQ Deca ion trap mass spectrometer (San Jose, CA, USA) equipped with an ESI interface. Mass spectra were acquired both in positive and negative mode. 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 as follows: capillary temperature 300 °C, capillary voltage 13 V, spray voltage 5 kV, sheath gas flow rate 35 (nitrogen, arbitrary units), auxiliary gas flow rate 10 (arbitrary units).

2.6. Assay of 2,2-Diphenyl-2-picrylhydrazyl (DPPH) Scavenging Activity

The antioxidant activity of the four *M. elaeodendroides* extracts was measured in terms of hydrogen-donating or radical scavenging ability, using the stable radical DPPH [24]. In the test tubes, 1.5 mL of DPPH (0.075 mg/mL) in ethanol was mixed with 750 µL of five concentrations of the different extracts to evaluate in a range of concentrations lower than 1000 µg/mL. A control sample (absolute ethanol) and reference (750 µL absolute ethanol and 1.5 mg/mL of DPPH solution) were also used. The decrease in the absorbance was determined at 515 nm, until the reaction plateau step was reached. α Tocopherol (Sigma, Fukushima, Japan) was used as an antioxidant standard. Three independent tests were performed for each sample. Then, the IC_{50} values (total antioxidant extract necessary to decrease the initial DPPH radical concentration by 50%) were determined. The inhibition percent of $DPPH^{\bullet}$ radical was calculated by: Inhibition (%) = (D.O. control − D.O. sample)/D.O. control) × 100.

2.7. Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) assay is a method that measures the reduction of ferric ion ($Fe^{3+)}$ ligand complex to the intensely blue-colored ferrous (Fe^{2+}) complex by antioxidants in an acidic medium. Antioxidant activity is determined as increase in the absorbance at 593 nm, and the results are expressed as micromolar Fe^{2+} equivalents or relative to an antioxidant standard [25]. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at 10:1:1 (*v*/*v*/*v*). The reagent (3.4 µL) and sample solutions (100 µL) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 30 min. A solution of ascorbic acid (100 µM) was used as a standard. All solutions were used on the day of preparation. The results were expressed as µmol ascorbic acid equivalent/g extract. Analyses were performed in triplicate on each extract.

2.8. Statistical Analysis

Values were expressed as the mean ± standard error of the mean (SEM). Statistical analyses were performed with GraphPad Prism 5.0 (GraphPad, La Jolla, CA, USA). For multiple comparisons, a one-way ANOVA test was used followed by Bonferroni test a posteriori. Values of $p < 0.05$ were considered statistically significant.

2.9. Anti-Inflammatory Assay

2.9.1. Animals

The experiment was run in accordance with Good Laboratory Practice rules and animal protection laws. The experiment was approved by the ethical committee of Oriente University, which follows the guidelines from the Cuban Animal Ethical Committee. Male OF-1 mice, 20–22 g weight, were used. They were supplied by the National Center for Production of Laboratory Animals (CENPALAB), Havana City, Cuba, and were kept in standard laboratory conditions with water and food ad libitum. The mice had an acclimatization–quarantine of 7 days and remained under controlled temperature (21–24 °C), humidity (60–65%), and alternative light/dark cycle of 12 h.

2.9.2. Experimental

For the determination of the anti-inflammatory activity, the edema method induced by croton oil in the mouse ear was used [26]. Different doses of the extracts (ME-1, 2, 3, 4) were administered topically in the ear of the animals 1 h before the application of the croton oil (2 mg/ear in 20 µL of acetone). The doses evaluated were 0.5, 1.0, 2.0, and 4.0 mg/20 µL of acetone. The left ear of each animal (control) received the vehicle (acetone 20 µL). A group treated with indomethacin (3 mg/20 µL acetone) was used as the reference group. The inflammation was followed for five hours after the croton oil application. After this period, the animals were sacrificed and immediately a 6 mm diameter disc from each ear was removed and weighed. The inflammation induced by croton oil was quantified as the

increase in weight of the biopsy of the ears (right) treated, minus the left (untreated) ears, as weight of the edema.

3. Results and Discussion

3.1. Isolation and Structure Elucidation of Major Compounds of EtOAc Extract

The EtOH extract of the stem bark of *M. elaeodendroides* was concentrated by reduced pressure and extracted with EtOAc and *n*-BuOH in a separating funnel. The EtOAc extract was subjected to Sephadex LH-20, silica gel column chromatography (CC), and preparative thin layer chromatography to yield compounds **1**–**4** (Figure 1). The structures of the compounds present into the extract were elucidated based on 1D and 2D NMR spectra, ESI-MS data and by comparing the obtained data with previously published values.

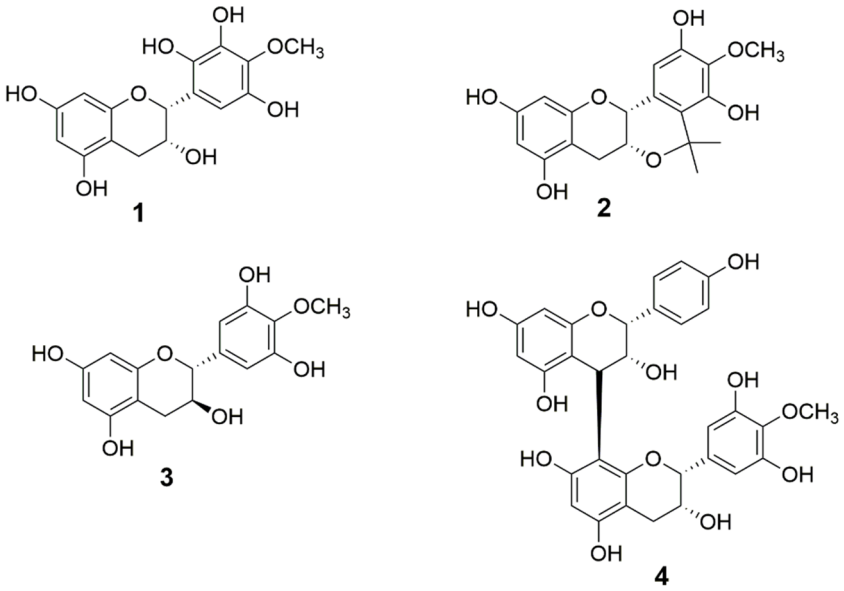

Figure 1. Compounds **1**–**4** isolated from *M. elaeodendroides* stem bark.

Compound **1** was isolated as a red solid of melting point 160–161 °C (recrystallized acetone) and $[\alpha]^{20^\circ}{}_D = -3.7$ (c= 0.0029 mol/L, CH_3OH). The molecular formula $C_{16}H_{15}O_8$ was determined from the deuterated molecular ion peak at *m/z* 342.1222 in the positive ion ESI/HRMS measurement. The NMR-^{1}H spectrum (DMSO-d_6) showed a total of 8 signals corresponding to 10 protons, 3 of them appear in aromatic protons area clearly divided into two groups (Figure S1). This behavior is typical of a compound of flavonoid nature. The characteristics of the region between 2.40 and 5.00 ppm and the doublets at 5.72 ppm (1H, d, 2.0 Hz, H-8) and 5.89 ppm (1H, d, 2.0 Hz, H-6) indicate a catechin derivative.

In the proton zone of ring B, a singlet was observed that integrates one proton (1H, s), so it was concluded that there are 4 substituents in the ring. In addition, it was observed 1 singlet that integrates 3 protons at 3.66 ppm correspond to a methoxyl group bound to an aromatic ring, so one of the substituents of ring B must be a methoxyl group.

The existence of two doublets of doublets in the most armored zone of the spectrum at 2.48 ppm (1H, dd, 3.5 Hz, 16 Hz, H-4e) and 2.68 ppm (1H, dd, 4.5 Hz, 16 Hz, H-4a) indicate the presence of two protons attached to the same carbon (C-4). Also, the zone between 4 and 5 ppm shows a broad singlet at 4.68 ppm (1H, brs; H-2) that integrates a proton and a doublet at 4.10 ppm (1H, d, 3 Hz, H-3) corresponding to a proton attached to a carbon with an electroacceptor substituent. Chemical shifts and the appearance of these two last signals are characteristic of 2,3-cis-flavan-3-ol [27]. All of this indicates that compound **1** is an epicatechin derivative. The coupling between the protons H-2, H-3, H-4a, and H-4e was confirmed with the help of the two-dimensional COSY experiment (Figure S2).

The two-dimensional experiments HSQC and HMBC (Figure S3) allowed us to unequivocally assign all the signals (Table S1). The HSQC experiment showed a coupling between the B ring proton at 6.41 ppm and the carbon at 105.95 ppm, (Figure S4), indicating that both were directly linked. In addition, there was correlation to two or three bonds (HMBC experiment) between this carbon and the proton H-2 (4.68 ppm), which places the proton of ring B in the 6′-position.

In the HMBC experiment, a correlation was observed between the proton H-6′ and a carbon whose chemical shift (134.4 ppm) was comparable to those observed for other catechins that present a methoxyl group in an aromatic carbon; therefore, this carbon can be found in positions 2′, 4′, or 5′. The absence of an NOE effect in the NOESY-1D experiment (Figure S5) affirms that the methoxyl group is bound to carbon 4′. According to all the evidence observed in the spectroscopic analysis, compound **1** was identified as 2′-hydroxy-4′-methoxy-epigallocatechin, isolated from a natural source for the first time.

Compound **2** corresponded to elaeocyanidin, which was reported for the first time in *Elaeodendron balae* root bark by Weeratunga G. et al. in 1985 [28]. Compound **3** was identified as 4′-*O*-methylgallocatechin. The 2,3-*trans* configuration was obvious from the large coupling constant (6 Hz) observed between H-2 and H-3. This compound was isolated for the first time from *Panda oleosa* [29]. It has been also isolated from *Stryphnodendron obovatum* [30], *Parapiptadenia rigida* [27], *Elaeodendron schlechteranum* [28], and between other plants. Compound **4** was identified as afzelechin-(4β->8)-4′-*O*-methyl epigallocatechin, reported from *Ouratea* spp. [31]. In this compound, H-2 on rings C and F occurs as a broad singlet, which is in agreement with a small coupling constant (<2 Hz) between H-2 and H-3. The 3,4-*trans* configuration in the C ring was established by the broad singlet appearance of the H-3 and H-4 signals [32]. The afzelechin-(4β->8)-4′-*O*-methyl epigallocatechin has been identified in *Prionostemma aspera* and *M. rigida* [33] and in the root bark of *Elaeodendron balae* [28].

3.2. FIA/ESI/IT/MS^n Analysis

The qualitative chemical composition of the EtOAc extract from stem barks of *M. elaeodendroides*, was analyzed by FIA/ESI/IT/MS^n. Negative mode spectra were selected for their better sensitivity. The total ion mass spectra of EtOAc extract (Figure 2) shows the $[M\text{-}H]^-$ ions of the major secondary metabolites present in the extract. Analysis of this spectrum and ESI/IT/MS^n experiments suggested the presence of deprotonated molecules of 2′-hydroxy-4′-methyl-epigallocatechin (*m/z* 335; **1**), elaeocyanidin (*m/z* 359; **2**), 4′-methyl epigallocatechin (*m/z* 319; **3**), and the monomers epigallocatechin (*m/z* 305) and epicatechin (*m/z* 289). The MS^2 of these ions show characteristic fragments of flavan-3-ol compounds, which represent C-ring cleavage through a retro-Diels–Alder (RDA) mechanism, loss of water with double-bond formation, and loss of B-ring. Product ions formed by loss of B-ring and RDA fragmentation indicate the methyl group position in compounds **3**. The stereochemistry of C3 on the flavan-3-ol cannot be determined by mass spectrometry [34]. Also, a dimer afzelechin-(4β->8)-4′-O-methyl epigallocatechin (*m/z* 591, **4**) and other oligomers were detected. Deprotonated ions at *m/z* 365, 639, 911, 959, and 1183 were identified as adducts of ions at *m/z* 319, 593, 865, 913, and 1135, respectively, with formic acid. The sequence of detected oligomers was determined through the three main fragmentation pathways described for proanthocyanidins: retro-Diels–Alder (RDA) fission, heterocyclic ring (HRF) fission, and quinone methide (QM) fission [34]. Mass spectrometric techniques cannot provide information about the position and stereochemistry of the interflavanoid linkage (4-6) or (4-8). It has been reported that proanthocyanidins with 4-8 linkage are stereochemically favored and require fragmentation energies lower (30%) than proanthocyanidins with 4-6 linkage [34,35] (60%). Thus, the compounds identified in the extract were assigned as presented in Table 1.

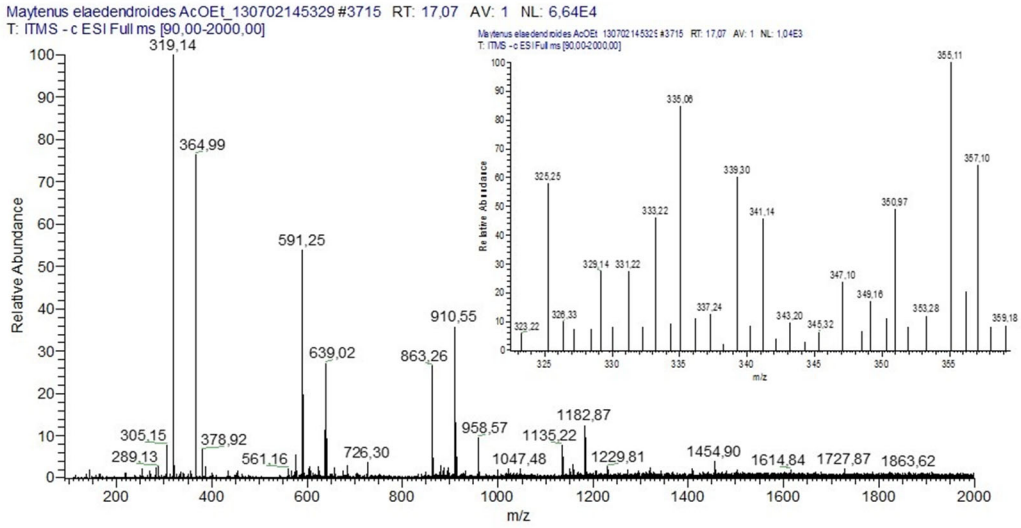

Figure 2. Total ion mass spectra of EtOAc extract from stem bark of *Maytenus elaeodendroides*.

Table 1. Monomers and oligomers identified in EtOAc extract from stem bark of *Maytenus elaeodendroides*.

[M-H]$^-$ *m/z*	MS2 Main Fragments	Proposed Names
	Monomers	
289	271, 137	(E)C
305	287, 137	(E)GC
319	301, 137	4-methyl-(E)GC (3)
335	317, 137	2′-hydroxy-4′-methyl-(E)GC (1)
359	341, 137	Elaeocyanidin (2)
	Dimers	
561	527, 425, 419, 407, 271, 289	(E)AZ-(E)C
577	559, 451, 425, 407, 289, 287	(E)C-(E)C
591	573, 465, 455, 437, 271, 319	(E)AZ-4´-methyl-(E)GC (4)
593	575, 467, 441, 423, 287, 305	(E)C-(E)GC
	Trimers	
863	845, 737, 591, 271	(E)AZ-(E)AZ-4´-methyl-(E)GC
865	739, 713, 695, 577, 425	(E)C-(E)C-(E)C
913	787, 609, 607, 305, 303	(E)GC-(E)GC-(E)GC
	Tetramer	
1137	863, 849, 575, 561,	(E)C-(E)C-(E)C-(E)AZ

Abbreviation: (E)C: (epi) catechin, (E)GC: (epi) gallocatechin, (E)AZ: (epi) afzelechin.

3.3. Antioxidant and Anti-Inflammatory Activities

The interest in health benefits of polyphenols has been well associated with their antioxidant and free radical scavenging effects. There is evidence that supports a contribution of polyphenols to the prevention and control of different diseases [36,37]. The latest advances in the role of diet in modulating gut microbiota, for example, have suggested a new phase of food bioactives research along the phytochemicals–gut microbiota–intestinal metabolites and low-grade inflammation–metabolic syndrome axis [38,39]. Plants used in traditional medicine can provide diverse secondary metabolites with bioactive activities, where the antioxidant potential related to the presence of phenolic compounds is relevant [35].

The assay of scavenging the stable DPPH radical permit evaluation of the free radical scavenging ability of compounds [40]. In this case, the antioxidant effect of the analyzed sample on DPPH radical scavenging may be due to their hydrogen donating ability and to reduce the stable violet DPPH radical to the yellow DPPH-H. Substances which are able to perform this reaction can be considered as antioxidants and, therefore, radical scavengers. On the way, the FRAP assay is based on the ability of antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of tripyridyltriazine (TPTZ), forming the intense blue Fe^{2+}–TPTZ complex, then the absorbance increase is proportional to the antioxidant content [41]. Here, we evaluated the antioxidant potential of the four extracts obtained from *M.s eleaodendoides*, an endemic specie of Cuba, by use of DPPH and FRAP methods.

The percentage inhibition of DPPH activity of four extracts obtained from *M. eleaodendoide* stem bark and the dose–response curves of the sequestering capacity of DPPH are shown in Figure S5. As can be seen, the highest percentage of inhibition of the radical coloration (indicative of the sequestering capacity) was for the EtOH extract (ME-1) (76%), reached from a concentration of 1000 µg/mL. This is in accordance with the IC_{50} value, which was in the order of 6.23 µg/mL (Figure S6). It was expected as this extract is obtained using ethanol as a solvent in the extraction process, a compound of high polarity that favors the extraction of phenolic compounds, metabolites with recognized antioxidant activity [38]. Meanwhile, the EtOAc extract (ME-2) and *n*-BuOH extract (ME-3) also showed significant DPPH radical reduction capacity, showing maximum inhibition values of 71% and 68%, respectively. In the case of the diethyl ether/petroleum ether 1:1 extract (ME-4), although it could reduce the DPPH radical, it showed the lowest activity (IC_{50} = 137.7 µg/mL), showing an inhibition percentage in the order of 62% at the concentration of 2000 µg/mL (Table 2). The IC_{50} values of the ME-1, ME-2, and ME-3 extracts were significantly lower ($p < 0.05$) than the one of a tocopherol, the positive control of the experiment. A similar trend in FRAP values was observed among the four extracts of *M. eleaodendoide* evaluated, showing the highest ability to reduce Fe^{3+} to Fe^{2+} for ethanolic extract ME-1 (3156 µmol ascorbic acid equivalent/g) (Table 2).

Table 2. Antioxidant activity of *Maytenus elaeodendroides* stem bark extract. Equal letters indicate no significant differences.

Extracts	FRAP (mM of Ascorbic Acid Equivalents Per Gram of Extract)	DPPH IC50 (mg/mL) (% Maximal Inhibition)
ME-1	3156 ± 753 a	6.23 a ± 1.5 (76)
ME-2	2983 ± 675 a	18.71 b ± 4.3 (71)
ME-3	2865 ± 666 a	10.44 b ± 3.5 (68)
ME-4	2434 ± 537 b	137.7 c ± 6.1 (62)
α Tocopherol	-	38.04 d ± 1.2 (77)

Compounds identified in *M. eleaodendoide* extracts are known to possess antioxidant activity. Previous studies revealed that the antioxidant effectiveness of proanthocyanidins increases with the degree of polymerization and the number of free hydroxyls [42]. According to that, polymeric proanthocyanidins have been verified to exhibit the highest antioxidant activities [43]. Then, the antioxidant capacity observed for the extracts could be associated with the presence of these bioactive compounds.

Values represent the mean ± SEM of ascorbic acid equivalents per gram of *M. elaeodendroides* extracts, as the capacity to reduce Fe^{3+} to Fe^{2+}. IC_{50} values were calculated as the extract concentration required to scavenge 50% of DPPH•; α-Tocopherol was used as standard for the DPPH• assay. Different letters represent statistical differences between the products (ANOVA, Bonferroni test a posteriori, $p < 0.05$). Three independent assays were performed and samples were analyzed in triplicate.

Inflammation is a process involved in the pathogenesis and progression of many diseases. It is a physiological response that protects the body against tissue damage or microorganisms [44]. The inflammatory response serves as a defense tool for the organism, but if it occurs in an exacerbated way, different pathological disorders can take place [45]. For suppressing the inflammatory reaction, it is necessary to use anti-inflammatory drugs. The conventional anti-inflammatory drugs used in clinical practice usually have adverse side effects, making it necessary to search for new alternative substances [46]. In this way, natural products can be an important source for the development of new therapeutic agents. Here, the extracts from *M. elaeodendoides* species were also evaluated to verify their possible anti-inflammatory effects in the model of edema induced by croton oil in the ear of the mouse (Figure 3). As our data showed, the four extracts tested prevented ear inflammation at doses of 4 mg/ear ($p < 0.05$) compared to the negative control (acetone). Meanwhile, the ME-1 extract showed the highest inhibitory effect. Indomethacin (3 mg/ear), a non-selective inhibitor of cyclooxygenase (COX) that reduces the production of prostaglandins, promoting pain and the inflammation, was used as a positive control of the experiment and as it was expected it produced a significant inhibition ($p < 0.05$) of edema (96%).

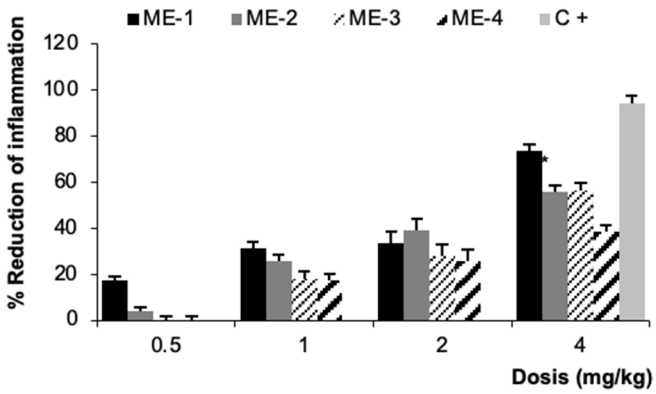

Figure 3. Anti-inflammatory effects of *M. elaeodendroides* extracts. Each bar represents the means ± ESM of seven animals per group. Values of * $p < 0.05$ represent statistically significant differences with respect to the control group. Positive control Indometacine (C+, 3 mg/ear).

M. elaeodendroides derivates have been used for many years in Cuban traditional medicine for the treatment of different inflammatory disorders [16]. In this study, we used a simple, but well stabilised in vivo model for verifying these properties. Topical application of phorbol esters (like croton oil) induces a long-lasting inflammatory response associated with a transient increase in prostanoid production and marked cellular influx [47]. Our results showed the four extracts at the higher doses tested attenuated the oedema induced by croton oil on the ear of the mice, suggesting anti-inflammatory activity. The inhibitory effects of epicatechin, a compound found in *M. elaeodendoides* extract, on lipopolysaccharide (LPS)-induced production of pro-inflammatory mediators in RAW264.7 cells have been shown [48]. Reports show epicatechin could down-regulate the expressions of inducible nitric oxide synthase and cyclooxygenase-2, as well as the production of nitric oxide, prostaglandin E2, and some pro-inflammatory cytokines in LPS-induced RAW264.7 cells. The authors correlated the attenuation of inflammatory responses by epicatechin with the inhibition of activation of an inhibitor of κB kinase α/β, the sequential translocation of nuclear factor-κB (NF-κB) p50/P65 subunits, to the suppression of activation of mitogen-activated protein kinases, Janus kinase 2 (JAK2)/signal transducer, and activator of transcription 3 (STAT3) [48].

The extracts could exert dual action due their observed antioxidant activity. It has been shown that the treatment of mouse skin with protein kinase C promoters, such as phorbol esters, promotes the formation of free radicals. Reactive oxidative species (ROS) are also relevant for the synthesis of some inflammation mediators. It is known that ROS

may regulate the production of TNF in the inflammatory response [49]. *M. elaeodendroides* extracts, particularly the ME-1 extract, could decrease tissue damage caused by hydrolytic enzymes and by some oxidant species.

4. Conclusions

In this study, the isolation of the 2′-hydroxy-4′-methoxy-epigallocatechin is reported for the first time. This study provides phytochemical information and data about the bioactive effects of stem bark from *Maytenus elaeodendoides* extracts grown in Cuba and emphasizes the rationale for using medicinal plants in folk medicine. The activities found for *M. elaeodendoides* extracts, particularly, in the ethanolic extract, may validate the traditional use of the plant in the treatment of health problems that derive from the consequence of oxidative stress and the inflammation process in the organism. Meanwhile, further tests are needed before this plant species and its metabolites can be considered as new therapeutic agents.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16110694/s1, Figure S1. NMR ^{1}H spectrum of compound **1**; Figure S2: COSY experiment of compound **1**; Figure S3: gHMBC experiment of compound **1**; Figure S4: gHMQC experiment of compound **1**; Figure S5: NOESY-1D experiment of compound **1**; Figure S6: Dose–response curves for the scavenging capacity of the DPPH radical of the extracts from *Maytenus elaeodendroides* evaluated. Table S1. Chemical shifts and correlations observed for compound **1**.

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Institutional Review Board Statement: The experiment was run in accordance with Good Laboratory Practice rules and animal protection laws. The experiment was approved by the ethical committee of Oriente University, which follows the guidelines from the Cuban Animal Ethical Committee.

Data Availability Statement: The original contributions presented in the study are included in the article and Supplementary Materials.

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