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Ellagic Acid Glucosides Detected By Lc-Ms In Ethanolic Branches And Fruit Extracts Of *Eugenia Pisiformis* Cambess. (Myrtaceae)

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Abstract: *In Brazil, species Eugenia pisiformis Cambess (Myrtaceae) occurs in the Atlantic Forest, showing occurrence records in the states of Rio de Janeiro, Espírito Santo, Maranhão and Bahia. This species is an abundant bush, presenting discolored green leaves, crossed opposite, green calyx of flowers, corolla and white stamens, ripe fruit being orange yellow colored. A specimen of E. pisiformis was collected in Itatiaia National Park, State of Rio de Janeiro and its branches and fruits were dried, crushed and subjected to cold extraction by maceration with ethanol. LC-MS analysis of these extracts showed the presence of a mixture of glycosylated derivatives of ellagic acid in their chemical composition. Ellagic acid is well-known for its antimicrobial, antitumor and antioxidant properties which have been broadly reported in the literature. Hence, fruits and branches of this plant might be a potential source of this pharmacologically important secondary metabolite and its derivatives.*

Keywords: *Eugenia pisiformis, LC-MS, ellagic acid, bioactive compound.*

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I. Introduction

Among the genera belonging to the family Myrtaceae, Eugenia L. has approximately 1,009 species of neotropical distribution. They are spread from Mexico and Florida to the northwest of Argentina, and, in Brazil, it occurs mainly in the Midwest and Southeast regions, with species of trees and shrubs [1,2,3,4]. Species of this genus accumulate mainly phenolic substances, including flavonoids, catechins and tannins, as well as terpene substances. The high concentration of phenolic compounds is associated with high antioxidant activity, present in the species of this genus [5]. Due to the large number of species belonging to the genus Eugenia L., many of them are used in folk medicine as medicinal plants and studies have proven their antioxidant [6], antimicrobial [7, 8], antinociceptive [9] and anti-inflammatory[10] activities among several others. Branches and fruits of a specimen of *Eugenia pisiformis*, an abundant bush that occurs in some states of Brazil, were collected in the state of Rio de Janeiro. Their ethanolic extracts were analyzed by LC-MS, in a search for pharmacologically important natural compounds which would allow to state this plant as a natural source of these substances. A previous phytochemical study of *E. pisiformis* reported the presence of biologically active triterpenes in the extract of its leaves [11].

II. Material And Methods

1.1. Plant material and preparation of the extracts

Branches and fruits of *E. pisiformis* were collected and identified by Dr. Adriana Quintella Lobão, adjunct professor at Universidade Federal Fluminense, Rio de Janeiro, Brazil, at the National Park of Itatiaia, state of Rio de Janeiro, Brazil, in March, 2015. A voucher specimen is deposited at the Herbarium of the Botanical Garden of Rio de Janeiro under the code AL1468. Fresh plant material (1.05 Kg of branches and 850 g of fruits) was dried in an oven under the temperature of 40º C for 48 hours. Dried material was grounded to a fine powder in a mill and this powder was submitted to an extraction by percolation with cold ethanol for 10 days. Ethanol was further evaporated under vacuum and the extracts yielded 2.2g and 1.9g for the branches and fruits, respectively. Ethanol used for extraction was USP grade.

1.2. LC-MS/MS analysis of the plant extracts

Ethanolic extracts of branches and fruits were analyzed by LC-MS/MS at the Anayltical Center of the Instituto de Pesquisa de Produtos Naturais of the Universidade Federal do Rio de Janeiro, Rio de Janeiro city, in Brazil. 5 mg of the extracts were ressuspended in 5mL methanol (HPLC grade) and filtered through a Sep-pak

cartridge to yield 1mg/mL solutions. 1 mL of each solution each was injected separatedly in a Shimadzu chromatograph equipped with a Phenomenex Luna® C18, 2□ (150 x 2mm) column. Mobile phase was a mixture methanol and formic acid 1% in several concentrations: 20% MeOH (1-4 min.) going to 80% MeOH (5-40 min) and 100% MeOH (41-45 min). UV wavelength used for the detection of the compounds was both 254 and 280 nm. Mass spectra of each peak of the chromatographic analysis were recorded at a Mass Spectrometer microTOFQ II (Bruker), using N_2 as nebulizer gas, 12 eV as energy of collision and 6 eV as the quadrupole energy. Spectra were recorded at negative mode.

III. Results And Observations

In order to establish a comparison of the chemical profile of the extracts fruits with the literature, the ethanolic fruits and branches extracts of *E. pisiformis* species were analyzed by LC-MS/MS. **Figures 1** and **2** show the chromatograms of the ethanolic extracts of fruits and branches, at the UV wavelengths of 254 and 280 nm.

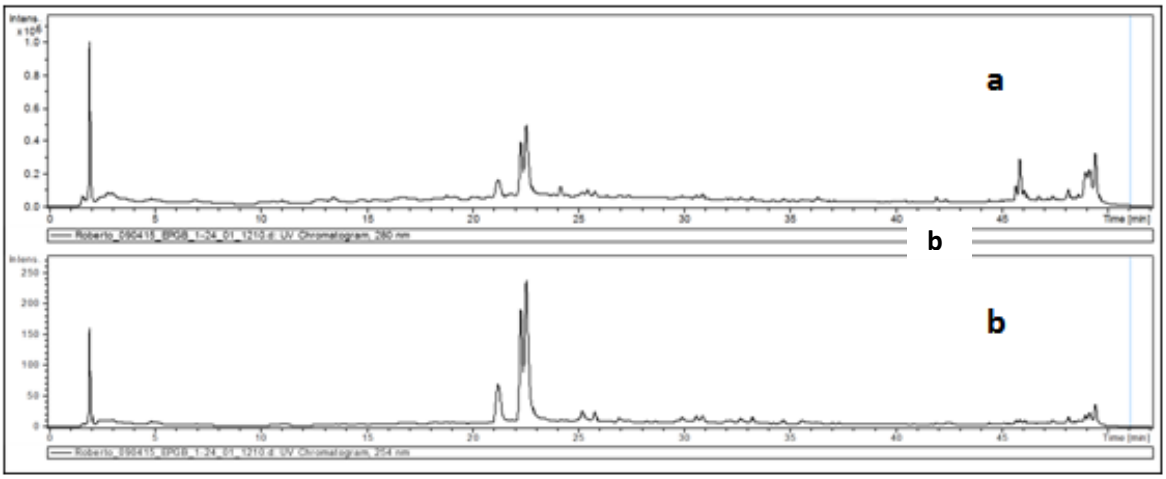


Figure 1. Chromatograms of ethanolic fruits extract using UV detection at 280 (**a**) and 254 nm (**b**).

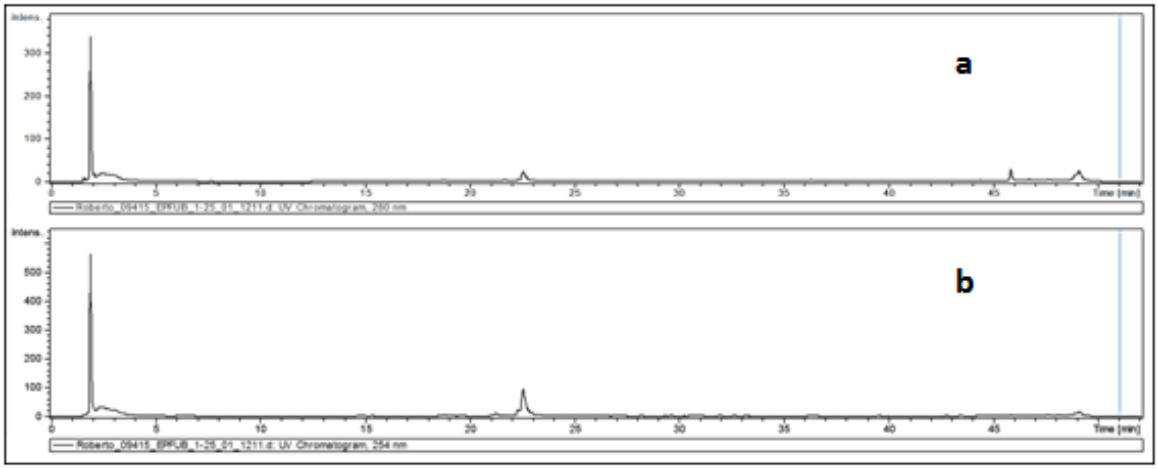


Figure 2. Chromatograms of ethanolic branches extract using UV detection at 280 (**a**) and 254 nm (**b**).

Mass spectra were obtained for each peak of the chromatograms. Data at the literature pointed out that in both were both extracts there is a mixture of ellagic acid derivatives, which have already been isolated from species of Eugenia [14, 15] but not yet found in *E. pisiformis*. The chromatogram of the ethanolic branches extract showed a signal with a retention time of 22.4 min. Analyzing the mass spectra for this signal (F**igure 3**) it is proposed that the ion of *m/z* 447 is precursor of the ion of *m/z 301*, resulting from the loss of an unit of rhamnoside (the deoxyhexose *m/z* 146) of a deoxyhexoside of ellagic acid (**Figure 4**). This hypothesis is supported by a previous study of branches extracts of *Eugenia uniflora* L[14].

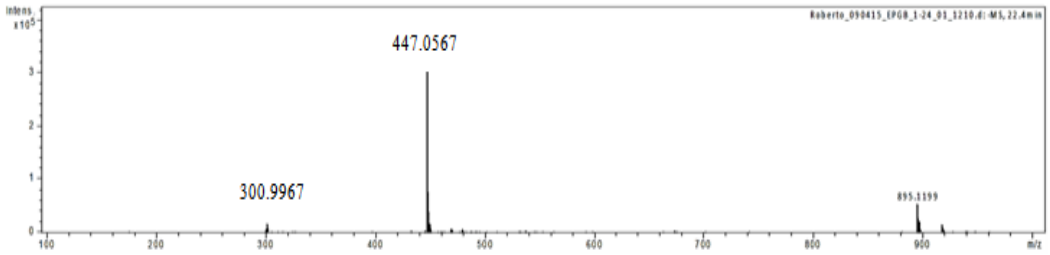


Figure 3. Mass spectrum of the signal at 22.4 min at the chromatogram of the ethanolic branches extract of E. Pisiformis

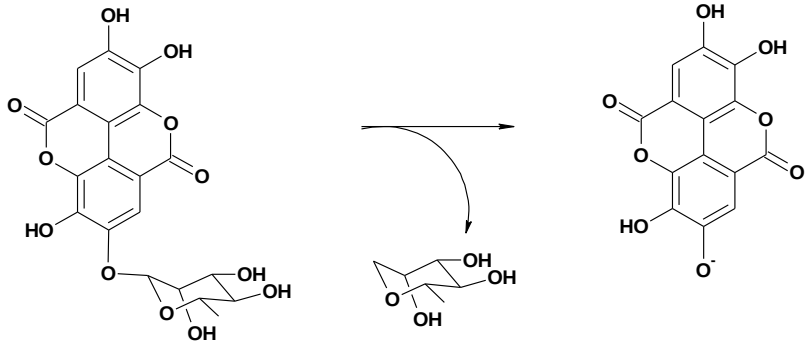


Figure 4. Fragmentation proposal for the ion M^{-H} 447 at the mass spectrum of the signal at 22.4 min in the chromatogram of the ethanolic branches extract of *E. pisiformis*, evidencing the loss of a desoxyhexoside residue to produce free ellagic acid.

Mass spectra in negative mode of the signals observed at 22.4 and 22.6 min (**Figures 5** and **6**, respectively) at the chromatogram of the ethanolic fruits extract also showed peaks in *m/z* 447 and 301, strongly supporting the evidence of these natural products in this sample as well. However, the structure of the deoxyhexoside residue for both substances might be different, what would be corroborated by deeper studies using other techniques, such as NMR experiments.

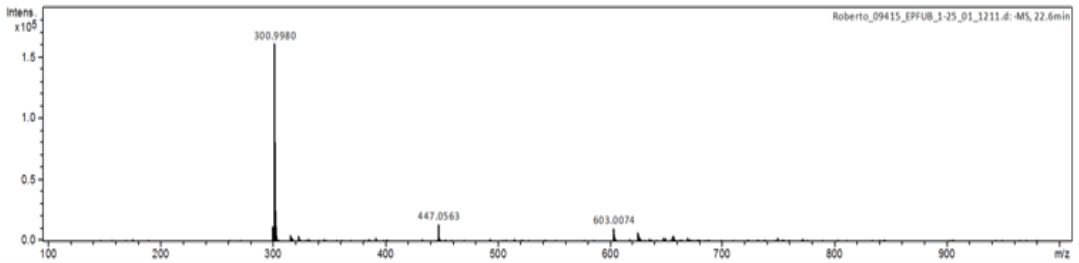


Figure 5. Mass spectrum of the signal at 22.4 min at the chromatogram of the ethanolic fruits extract of E. pisiformis

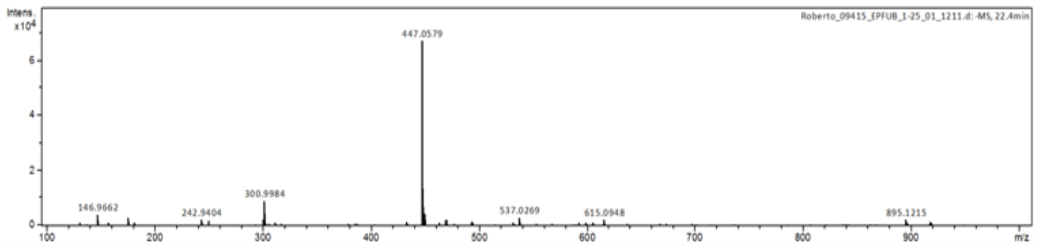


Figure 6. Mass spectrum of the signal at 22.6 min at the chromatogram of the ethanolic fruits extract of *E. pisiformis*

The signal with retention time of 21.3 min. in the chromatogram of the ethanolic extract of the branches presented a M^{-H} 433, which is supposedly the precursor of the ion at *m /z* 301 (**Figure 7**), resulting from the loss of a pentose (MW =132) of an ellagic acid pentoside (**Figure 8**) [16]. Once again, these preliminary results support the evidence that the substance at 21.3 min at the chromatogram is an ellagic acid pentoside,.

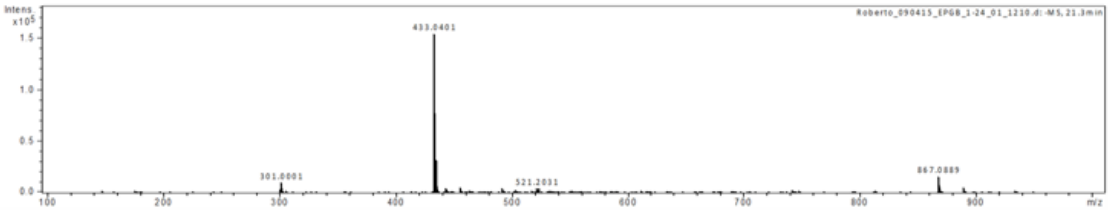


Figure 7. Mass spectrum of the signal at 21.3 min at the chromatogram of the ethanolic fruits extract of E. pisiformis

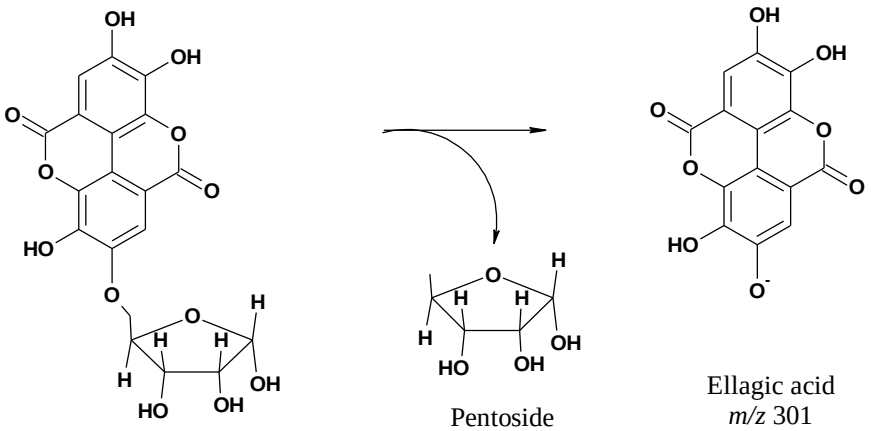
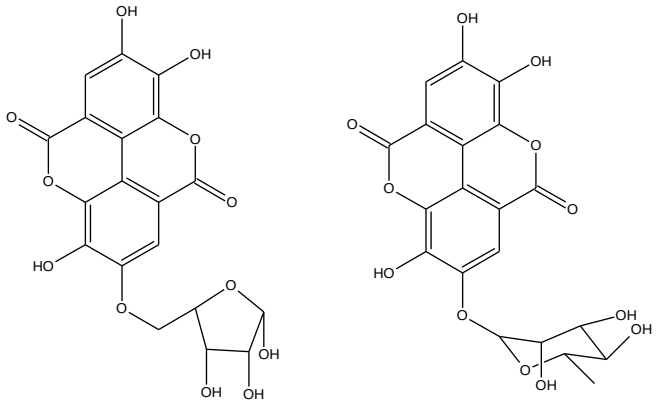


Figure 8. Fragmentation proposal for the ion M^{-H} 433 at the mass spectrum of the signal at 21.3 min in the chromatogram of the ethanolic branches extract of *E. pisiformis*, evidencing the loss of a pentoside residue (*m/z* 132) to produce free ellagic acid (*m/z* 301).

Based upon previous literature data, authors suggest that the substances might be 4-*O*-α-L-arabinofuranosylellagic acid and 4-*O*-α-L-rhamnopyranosylellagic acid (**Figure 9**) on which the sugar moieties are both linked to the oxygen at C4. These compounds have already been found in other species of Eugenia such as *E. uniflora*[14], as previously reported. However, more accurate analysis must be achieved by NMR experiments to properly confirm the identity of the structures detected on LC-MS.

Ellagic acid is a phenolic substance mainly present in grapes, pomegranates, raspberries, strawberries, hazelnuts, walnuts and nuts. Its chemical structure corresponds to a dilactone of gallic acid, which can be found in the free, glycosylated form and in the form of ellagitannins. It presents high antioxidant capacity, because of its four phenolic hydroxyl groups that can react directly with free radicals [12, 13]. Several biological activities of ellagic acid are described, such as antiproliferative activity and induction of apoptosis in culture of carcinogenic cells of the cervical epithelium [14,15], prevention of gastrointestinal tract cancer related to the selective accumulation of ellagic acid in rat epithelial cells [16] and selective antimicrobial potential in pathogenic microorganisms for humans [17]. In a review, Vatten and Shetty (2005) describe that ellagic acid can act as antimutagenic modulator of toxin metabolism, causing the inhibition of Phase I enzymes, responsible for the metabolism of xenobiotics; besides acting as anticarcinogenic, inhibiting the carcinogen binding to the DNA; as a potent inhibitor of DNA topoisomerases; as hepatoprotective, and as a profilaxy for animals poisoned by carbon tetrachloride [18].



4-*O*-a-L-arabinofuranosylellagic acid 4-*O*-a-L-rhamnopyranosylellagic acid

Figure 9. Structures suggested for the ellagic acid glucosides detected by LC-MS in the branches and fruits extracts of E. pisiformis

IV. Conclusion

As a second phytochemical study of *E. pisiformis*, it is possible to state that the fruits and branches of the species can be a source of potential bioactive compounds as ellagic acid and its derivatives. LC-MS, the instrumental technique used in this work, proved to be quick and reliable for this type of analysis. Ellagic acid and its glucosides have been also found in other species of Eugenia and literature reports several biological activities for these compounds.

References

[1]. Consolini AE, Baldini OAN, Amat AG. Pharmacological basis for the empirical use of *Eugenia uniflora* L. (Myrtaceae) as antihypertensive. Journal of Ethnopharmacology. 1999; 66: 33-39.

[2]. Magina, M. D. A. Estudo fitoquímico e biológico de espécies do gênero *Eugenia. pHD Thesis*. 2008. Universidade Federal de Santa Catarina, Florianópolis, Brazil.

[3]. Júnior, JEQF. O Gênero Eugenia L. (Myrtaceae) nos Estados de Goiás e Tocantins, Brasil. 2010. Masters Dissertation. 2010. Universidade de Brasília, Distrito-Federal, Brazil.

[4]. Kawasaki ML, Landrum L. A rare and potencially economic fruit of Brazil: Cambuci, *Campomanesia phaea* (Myrtaceae). Economic Botany. 1997; 51(4): 403-407.

[5]. Lucas EJ, Harris SA, Mazine FF, Belsham SR, Nic Lughadha EM, Telford AMW. A suprageneric phylogeny of tribe Myrteae (Myrtaceae) with biogeographical analysis and morphological discussion. Taxon. 2007; 55(4): 1105-1128.

[6]. Lenardão EJ, Savegnag, L, Victoria FN, Perin G, Jacob RG, Alves D, Silva WP, Motta AS, Nascente PS. Essential oil of the leaves of *Eugenia uniflora* L.: Antioxidant and antimicrobial properties. Food and Chemical Toxicology. 2012; 50 (8): 2668-2674.

[7]. Setzer WN, Ogunwande IA. et al. Studies on the essential oils composition, antibacterial and cytotoxicity of *Eugenia uniflora* L. International Journal of Aromatherapy. 2005; 15 (3): 147-152.

[8]. Chattopadhyay RR, Bag A, Bhattacharyya SK, Pal NK. *In vitro* antibacterial potential of *Eugenia jambolana* seed extracts against multidrug-resistant human bacterial pathogens. Microbiol. Res. 2012; 167(6): 352-357.

[9]. Amorim, ACL, Lima CKF, Hovell AMC, Miranda ALP, Rezende CM. Antinociceptive and hypothermic evaluation of the leaf essential oil and isolated terpenoids from *Eugenia uniflora* L. (Brazilian Pitanga). Phytomedicine. 2009; 16(10): 923-928.

[10]. Saha S, Subrahmanyam EVS, Kodangala C, Mandal, SC, Shastry SC. Evaluation of antinociceptive and anti-inflammatory activities of extract and fractions of *Eugenia jambolana* root bark and isolation of phytoconstituents. Brazilian Journal of Pharmacognosy. 2013: 23(4): 651-661.

[11]. Oliveira CA, Viana CB, Martins RCC. Biologically active triterpenes from *Eugenia pisiformis* Cambess (Myrtaceae). Int. J. of Pharm. Sci. and Drug Res. 2017; 9: 1-2.

[12]. Bianchi MLP, Antunes LMG. Free radicals and the main dietary oxidants. Rev. Nutr. Campinas. 1999; 12(2): 123-130.

[13]. Clifford MN, Scalbet A. Ellagitannins-nature, occurrence and dietary burden. J. Sci. Food Agric. 2000; 80: 1118-1125.

[14]. Oliveira AL, Destandau E, Fougère L, Lafosse M. Isolation by pressurized fluid extraction (PPE) and identification using CPC and HPLC/ESI/MS of phenolic compounds from Brazillian cherry seeds. Food Chem. 2014; 145: 422-529.

[15]. Narayanan BA, Geoffrey O, Willingham MC, Re GG, Nixon DW. P53/p21 (WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells. Cancer Letters. 1999; 136: 215-221.

[16]. Whitley AC, Stoner GD, Darby MV, Walle T. Intestinal epithelial cell accumulation of the cancer preventive polyphenol ellagic acid-extensive binding to protein and DNA. Biochem. Pharmacol. 2003; 66: 907-915.

[17]. Puupponen-Pimiã R, Nohynek L, Hartmann-Schmidlin S, Kähkönen M., Heinonen M, Määttä-Riihinen K, Oskman-Rangkadilok KM. Berry phenolics selectively inhibit the growth of intestinal pathogens. J Appl. Microb. 2005; 98: 991-1000.

[18]. Vatten DA, Shetty K. Biological functionality of ellagic acid: a review. J Food Biochem. 2005; 20: 234-266.

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