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Determination of guava (*Psidium guajava* L.) leaf phenolic compounds using HPLC-DAD-QTOF-MS

Elixabet Díaz-de-Cerio [a,b], *Ana María Gómez-Caravaca* [a,b], *Vito Verardo* [c,*], *Alberto Fernández-Gutiérrez* [a,b], *Antonio Segura-Carretero* [a,b]

[a] *Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avd. Fuentenueva s/n, 18071, Granada, Spain*

[b] *Functional Food Research and Development Center, Health Science Technological Park, Avd. del Conocimiento, Bioregion building, 18100, Granada, Spain*

[c] *Department of Chemistry and Physics (Analytical Chemistry Area) and Research Centre for Agricultural and Food Biotechnology (BITAL), Agrifood Campus of International Excellence (ceiA3), University of Almería, Carretera de Sacramento s/n, E-04120 Almería, Spain*

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ABSTRACT

Markets of different countries have proposed guava tea infusions as a drink that can modulate the glycaemic index in blood. This property has been attributed to the phenolic compounds contained in guava leaves. However, phenolic profile of guava leaves is still not well-known. Based on this information, different ethanol/water mixtures were used to extract the phenolic compounds in guava leaves. Phenolic identification was carried out by HPLC-ESI-QTOF-MS in guava leaves from *pomifera* and *pyrifera* varieties; moreover, the antioxidant activities of the ethanolic extracts were determined by TEAC and FRAP methods. To sum up, seventy-two phenolic compounds were identified. To our knowledge, twelve of them were determined for the first time in guava leaves. The highest amount of phenolic compounds was found in EtOH/H_2O 80:20 (v/v) mixture. Furthermore, *pyrifera* var. showed higher concentration of phenolic compounds than *pomifera* var. (113.34 vs. 86.12 mg/g leaf d.w.) and also greater antioxidant capacity.

* *Corresponding author.* Department of Chemistry and Physics (Analytical Chemistry Area) and Research Centre for Agricultural and Food Biotechnology (BITAL), Agrifood Campus of International Excellence (ceiA3), University of Almería, Carretera de Sacramento s/n, E-04120 Almería, Spain. Tel.: +34 958 637 206; fax: +34 958 637 083.

E-mail address: vito.verardo@unibo.it (V. Verardo)

Chemical compounds: Casuarinin (PubChem CID: 157395); Castalagin (PubChem CID: 168165); Gallocatechin (PubChem CID: 65084); Gallic acid (PubChem CID: 370); Morin (PubChem CID: 5281670); Quercetin (PubChem CID: 5280343); Hyperin (PubChem CID: 5281643); Reynoutrin (PubChem CID: 5320863); Avicularin (PubChem CID: 5490064); Quercitrin (PubChem CID: 5280459); Guajaverin (PubChem CID: 5481224); Myrciaphenone B (PubChem CID: 183139).

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1. Introduction

Diabetic complications are now a global health problem without effective therapeutic approach. Hyperglycaemia and oxidative stress are important components for the development of diabetic complications due to an excessive production of free radicals (Singh, Kaur, Kishore, & Gupta, 2013). It is known that plants are a rich source of secondary metabolites that have been implicated in several therapeutic methodologies like flavonoids, alkaloids, terpenoids and tannins. Thus, for diabetic complications, an antioxidant treatment coupled with other approaches could be effective in ameliorating these complications (Scott & King, 2004).

Psidium guajava L. is a small tree native to Central America from Southern Mexico to Northern South America. It is popularly known as guava and belongs to the myrtle family (Myrtaceae). Today, guava tree has been distributed through many countries as a result of its capacity to grow in tropical and subtropical conditions (Morton, 1987).

Extracts from the leaves of this plant have traditionally been used in folk medicine around the world. They are mainly known for their antispasmodic and antimicrobial properties in the treatment of diarrhoea and dysentery, although they also exhibit antioxidant, hepatoprotection, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, anti-inflammatory and antinociceptive properties. Besides, they have extensively been used as a hypoglycaemic agent for diabetes, due to their high concentration of total phenolic compounds (Gutiérrez, Mitchell, & Solis, 2008).

Several authors (Haida, Baron, Haida, de Faci, & Haas, 2011; Wang, Jiao, Liu, & Hong, 2007) reported that the leaves of white (*P. guajava* L. var. *pyrifera*) and red guava (*P. guajava* L. var. *pomifera*) presented higher amounts of phenolic compounds with antioxidant activity compared with other vegetable species.

In the last years, traditional and advanced techniques have been applied to extract phenolic compounds from natural product matrices (Stalikas, 2007). Nantitanon, Yotsawimonwat, and Okonogi (2010) found that ultrasonication was the best method to extract phenolic compounds from guava leaves, followed by Soxhlet extraction and maceration. The same authors affirmed that ultrasound assisted extraction is also simpler, faster and cheaper than conventional extraction methods. Thus, in this study, pure ethanol and different ethanol:water mixtures were used to extract commercial guava leaves. The extracts were analysed and evaluated by HPLC-DAD-ESI-QTOF. Moreover, the phenolic content and the antioxidant capacity of *P. guajava* L. var. *pyrifera* leaf extracts were compared with those of *P. guajava* L. var. *pomifera*.

2. Experimental

2.1. Chemicals

Double-deionized water with conductivity lower than 18.2 MΩ was obtained with a Milli-Q system (Millipore, Bedford, MA, USA). Methanol LC–MS "optima" grade and acetonitrile were obtained from Fisher Scientific (Leicestershire, UK). Acetic acid, TPTZ (2,4,6-tripyridyl-S-triazine), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonate)], potassium persulphate, ferric sulphate and the standards namely gallic acid, catechin, ellagic acid, naringenin, quercetin and rutin were all from Sigma-Aldrich (Steinheim, Germany). Ethanol, sodium acetate, ferric chloride, and hydrochloric acid were obtained from Panreac (Barcelona, Spain).

2.2. Plant material

Commercial *P. guajava* L. leaves were used for the optimization of solvent extraction. Then, *P. guajava* L. var. *pyrifera* and *pomifera* harvested in Motril (Spain) (36°44′43″N 3°31′14″W) were collected. They were middle age intense green leaves and they were collected in February 2015. The environmental conditions had mean max/min temperature of 23/8 °C, precipitation of 0–0.8 mm, and saturated light duration that ranged from 9.45 to 10.40 h per day.

2.3. Guava leaves extraction

The phenolic compounds extraction was performed using an ultrasound bath and a mixture of ethanol:water 80/20 (v/v) as extractant solvent. Briefly, 0.5 g of air-dried and crushed guava leaves were extracted with 15 mL of solvent (×3) using a sonicator Branson B3510 for 10 min at room temperature. Then, samples were centrifuged for 15 min at 6000 rpm using a centrifuge to remove solids. The supernatants were pooled, evaporated and dissolved in 2 mL of methanol/water 1/1 (v/v). This solution was filtered through a 0.20-μm RC syringe filter and kept at –20 °C in amber vials until analysis to avoid degradation. The analysis were run in triplicate (n = 3) and results expressed as mg of phenolic content/g leaf dry weight (d.w.).

2.4. Preparation of standards

Phenolic standards of interest such as gallic acid, catechin, ellagic acid, naringenin, and rutin were used for quantification of phenolic compounds in guava leaf extracts. The standard stock solutions were prepared at 250 mg/L in methanol, except for ellagic acid, which was solved in water. Then, each solution was diluted from 50 mg/L to 0.01 mg/L.

2.5. HPLC-DAD-ESI-QTOF-MS analysis

Chromatographic analyses were performed using an HPLC Agilent 1260 series (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump, an online degasser, an autosampler and a thermostatically controlled column compartment, and a UV–Vis Diode Array Detector (DAD). The column was maintained at 25 °C. Phenolic compounds from *P. guajava* L. leaves were separated at room temperature using a method previously reported by López-Cobo, Gómez-Caravaca, Švarc-Gajić, Segura-Carretero, and Fernández-Gutiérrez (2015) with slight modifications. Briefly, a Poroshell 120 EC-C18 (4.6 mm × 100 mm, particle size 2.7 μm) (Agilent Technologies) was used to separate the compounds. The gradient elution was carried out using water containing 1% acetic acid as solvent system A and acetonitrile as solvent system B, and applied as