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Comprehensive characterization and antioxidant activities of the main biflavonoids of *Garcinia madruno*: A novel tropical species for developing functional products

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ABSTRACT

The aim of the study was to determine the qualitative and quantitative plant secondary metabolite profile of *Garcinia madruno* by LC-MS^n and to evaluate the structure–activity relationship (SAR) with its antioxidant properties. A total of 21 biflavonoids and 3 organic acids were identified. The leaves were the most promising source of biflavonoids, and the epicarp was the richest source of morelloflavone type biflavonoids (>10%). Morelloflavone and fukugiside were the major biflavonoids found in all samples as well as the compounds responsible for antioxidant activity. The inter-flavonoid bond did not increase the antioxidant activity by single electron transfer (SET) mechanism but increased the activity by single proton-loss electron transfer (SPLET) mechanism. The results suggest that *G. madruno* contains potentially useful amounts of bioactive biflavonoids; in particular, the leaves and epicarp may be a potential source of functional products.

1. Introduction

Garcinia is considered the most diverse and bountiful genus of the Clusiaceae family (Jamila, Khairuddean, Khan, & Khan, 2014). Phytochemical studies have reported the isolation and identification of biflavonoids, flavonoids, benzophenones, xanthones, and organic acids. As a chemotaxonomic particularity, some species of *Garcinia* overexpress specific metabolites from one of the mentioned groups. For instance, the benzophenones are the most representative secondary metabolites from *Garcinia xanthochymus*, *G. mannii*, *G. staudtii*, and *G. subelliptica* (Acuña, Dastmalchi, Basile, & Kennelly, 2012; Hemshekhar et al., 2011); xanthones are found in the fruits of *G. mangostana* (Wittenauer, Falk, Schweiggert-Weisz, & Carle, 2012); organic acids (specially hydroxycitric acid) are found in the fruits of *G. cambogia* (Semwal, Semwal, Vermaak, & Viljoen, 2015); and biflavonoids are found in the seeds of *G. kola* (Ayepola,

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Cerf, Brooks, & Oguntibeju, 2014), the fruits of *G. brasiliensis* (Gontijo et al., 2012) and the cortex of *G. hombroniana* (Jamila et al., 2014). A compilation of scientific data shows that many of those species have been exploited in pharmaceutical and food fields. In fact, *G. mangostana, G. cambogia* and *G. kola* are the most recognized *Garcinia* species used in nutraceutical, dietary supplements or functional foods products. The use of these species have been reported for the prevention or treatment of multiple symptoms and diseases such as ulcers, diarrhoea, hypertension, obesity, inflammatory disorders, hepatic damage, among others, and it has been related mainly to the content of biflavonoids, hydroxycitric acid, xanthones and benzophenones (Adegbehingbe et al., 2008; Farombi, 2011; Hemshekhar et al., 2011; Saiyed et al., 2015; Tang et al., 2009; Udani, Singh, Barrett, & Singh, 2009; Vasques et al., 2014). Meanwhile, *G. madruno* (Kunt) Hammel (known as "madroño") is a tropical tree of Central and South America characterized by its exotic yellow fruit with edible pulp of mangosteen-like bittersweet flavour (Cury, Abela, Bravo, Peñarrieta, & Rendón, 2012). Previously, we isolated and identified from the leaves some known biflavonoids: amentoflavone, morelloflavone, volkensiflavone, fukugiside and espicataside, as well as madrunoudeaside, a novel acetylglucoside of morelloflavone (Osorio, Londoño, & Bastida, 2013; Osorio, Montoya, & Bastida, 2009).

Biflavonoids are characterized as the covalent union of two monomeric units of flavonoids through C—C or C—O—C bonds between flavanone–flavone, flavone–flavone, flavanone–flavanone or flavone–flavanonol (Ferreira, De Carvalho, & Da Silva, 2012). At the same time, those monomers may present several substitutions, giving rise to glycosylated, methylated, sulphated or isoprenylated biflavonoids, among others (Acuña et al., 2010; Ito et al., 2013; Yang et al., 2010). Therefore, a theoretical number of biflavonoids could exist. However, unlike some of their monomeric constituents, biflavonoids are characterized by presenting a distribution among nature restricted to some species, highlighting the *Ginkgo biloba* and some species from the genera *Garcinia* and *Selaginella* (Ferreira et al., 2012; Kim, Park, Son, Chang, & Kang, 2008). The *Garcinia* biflavonoids generally lead to 3→8″ biflavanones or 3→8″ flavanone–flavone and carry at least one stereogenic centre but also show atropisomeric behaviour due to restricted rotation about the central axis (Li et al., 2002; Osorio et al., 2013). Nonetheless, the majority of the reports regarding these biflavonoids involve laborious pre-treatment and methodological approaches based on chromatographic isolations and identification by spectroscopic techniques. Thereupon, the results obtained from these methodologies, while confirming the presence of a series of metabolites, their limitations and the lack of quantitative strategies have not allowed determination of the metabolic profiles in relation to the expression and content of biflavonoids, preventing us from performing qualitative–quantitative comparisons intra- and inter-species, as well as establishing the main natural sources of these compounds.

A number of studies have shown that biflavonoids generally possess a high level of antioxidant activity and relevant pharmacological activities such as being antimicrobial, antiallergenic, anti-inflammatory, hepatoprotective and antiviral (Ferreira et al., 2012). In relation to the *G. madruno* biflavonoids, previous studies have shown that these compounds have the ability to inhibit the lipid peroxidation of the human LDL and to stabilize the DPPH• radical (Osorio et al., 2009, 2013). Morelloflavone has also been associated with hypocholesterolemic (Tuansulong, Hutadilok-Towatana, Mahabusarakam, Pinkaew, & Fujise, 2011), anti-inflammatory (Otuki et al., 2011) and atheroprotective (Decha-Dier & Hutadilok-Towatana, 2008; Pinkaew et al., 2009; Pinkaew, Hutadilok-Towatana, Teng, Mahabusarakam, & Fujise, 2012) activities. The chemical and antioxidant profiles of *G. madruno* biflavonoids may point to a better understanding of the role of these substances in physiology processes. However, although the functionality of biflavonoids is highly related to their antioxidant capacity, no clear comparison has been made to understand the structural differences among biflavonoids with the antioxidant activity or that compares the activity between biflavonoids and their respective flavonoids monomers. Therefore, the aim of the present study was to provide a comprehensive antioxidant activity and a qualitative and quantitative characterization of the *G. madruno* biflavonoids using a metabolomics approach based on HPLC–DAD–MS^n and a SAR analysis.

2. Material and methods

2.1. Chemical and reagents

All solvents used were analytical or HPLC grade (Merck Chemicals, Darmstadt, Germany). Deionized water was obtained with a Mill-Q water purification system (Millipore, Bedford, MA, USA). Standard compounds (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylicacid (Trolox), naringenin, apigenin, luteolin, luteolin-7-O-glucoside, quercetin and (+)-catechin were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The reference standard amentoflavone and biapigenin were purchased from TCI Chemical Co. (Tokyo, Japan) and Phytolab (Vestenbergsgreuth, Germany), respectively; whereas morelloflavone, fukugiside and volkensiflavone were isolated from a biflavonoid fraction previously obtained (Osorio et al., 2013) using an HPTLC methodology (Camag-Linomat 5, Switzerland). The chromatography purity of the isolated standard compounds was >97% for each compound. The reagents 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), fluorescein and 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) were obtained from Sigma Chemical Co. (St. Louis, MO, USA); Na_2HPO_4 and KH_2PO_4 were purchased from Carlo Erba reagents (Milan, Italy); and sodium acetate and $FeCl_3$ were obtained from J.T. Baker (Xalostoc, México).

2.2. Plant material

Five different *G. madruno* samples were used: (1) leaves, (2) stems, (3) epicarp, (4) mesocarp and (5) seeds. All samples were collected in February of 2014 from trees located on the Antioquia University campus. Samples 1 and 2 were dried at 40 °C for five days in a drying oven, and samples 3, 4 and 5 were frozen with liquid nitrogen and freeze-drying (EYELA Freeze Dried FDU-1200). All dried samples were mechanically blended until obtaining a homogeneous particle size. Finally, all the powdered samples were stored at room temperature, protected from light and moisture.