Journal of King Saud University – Science 31 (2019) 1283–1289

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Chemical constituents and antioxidant activity of *Garcinia madruno* (Kunth) Hammel

Cesar Ramirez [a], Jesús H. Gil [b], J. Camilo Marín-Loaiza [c,*], Benjamín Rojano [a], Diego Durango [a]

[a] Universidad Nacional de Colombia-Sede Medellín, Grupo de Química de los Productos Naturales y los Alimentos, Facultad de Ciencias, Escuela de Química, Calle 59ª 63-020 Autopista Norte, AA 3840, Medellín, Colombia
[b] Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Departamento de Ingeniería Agrícola y Alimentos, Calle 59ª 63-020 Autopista Norte, AA 1779, Medellín, Colombia
[c] Universidad Nacional de Colombia-Sede Bogotá, Grupo de investigación en fitoquímica y farmacognosia de la Universidad Nacional de Colombia (GIFFUN), Facultad de Ciencias, Departamento de Farmacia, Colombia

ARTICLE INFO

Article history:
Received 23 January 2018
Accepted 22 July 2018
Available online 24 July 2018

Keywords:
Antioxidant
Garcinia madruno
Biflavonoids
Garcinol
Morelloflavone

ABSTRACT

Leaves, epicarp and seeds of *Garcinia madruno* (Kunth) Hammel were extracted by percolation using consecutively *n*-hexane, dichloromethane, ethyl acetate, and methanol until exhaustion. Then, fractions were screened for their phenolic content (Folin-Ciocalteu method) and antioxidant properties (DPPH, ABTS, FRAP methods). The phenolic content ranged from 2404 ± 121 (hexane fraction) to 9097 ± 275 mg GAE/100 g d.w. (ethyl acetate fraction) for seeds, 2372 ± 153 (dichloromethane fraction) to 11292 ± 962 mg GAE/100 g d.w. (ethyl acetate fraction) for epicarp, and 3659 ± 180 (dichloromethane fraction) to 23510 ± 255 mg GAE/100 g d.w. (ethyl acetate fraction) for leaves. The antioxidant activity was significantly depending on the nature of the extracting solvent and the organ of the plant. The highest values of antioxidant activity were displayed for ethyl acetate and hexane fractions. For ABTS assay, ethyl acetate fraction reached 57069 ± 1350, 67768 ± 2990, and 377848 ± 6039 mg AAE/100 g d.w. for seeds, epicarp, and leaves, respectively. From the most active fractions (ethyl acetate and hexane), three compounds were isolated by chromatographic techniques and their structures were elucidated by spectroscopic methods corresponding to the biflavonoids morelloflavone and volkensiflavone (from ethyl acetate fraction); and the polyisoprenylated benzophenone garcinol (from hexane fraction), first time reported in this species. These metabolites were quantified by high performance liquid chromatography and their antioxidant properties (DPPH, ABTS, FRAP, ORAC) evaluated. The highest concentration of the biflavonoids and the polyisoprenylated benzophenone was found in ethyl acetate and hexane, respectively. The biflavonoid morelloflavone displayed the highest antioxidant activity with 209216 ± 11723 (ABTS) and 293842 ± 22026 (ORAC) μmol Trolox/100 g. The results suggested that *G. madruno* might be a good source of metabolites with antioxidant activity.

1. Introduction

Garcinia (Clussiaceae syn. Guttiferae), is a genus native from Asia and Africa with more than 300 species of trees and small bushes, which are mainly found in tropical lowland forest and characterized by latex production (Sweeney, 2008; Osman and Rahman, 2006). Many of the species belonging to this genus are edible and used as ornamental (Hemshekhar et al., 2011). Some *Garcinia* species, employed as medicinal plants, have been evaluated in a great number of biological and pharmacological assays; showing their great therapeutic potential (Dal Molin et al., 2012; Lin et al., 2012; Mackeen et al., 2012; Siridechakorn et al., 2012; Xia et al., 2012; Otuki et al., 2011; Elfit et al., 2009). Previous chemical studies have established the presence of different types of metabolites (phenols, terpenes, and alkaloids), prevailing xanthones and polyisoprenylated benzophenones (Hemshekhar et al., 2011).

Particularly, *Garcinia madruno* (Kunth) Hammel., is a tropical fruit tree commonly found in places bordering rainforests between 100 and 1000 mamsl. *G. madruno* is a native species from Centro and South America, traditionally known as "madroño", "charichuelo",

* Corresponding author.
E-mail address: jcmarinlo@unal.edu.co (J.C. Marín-Loaiza).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2018.07.017
1018-3647/

"cozoiba", "naranjita", "ocoró", "satro", among others (Lim, 2012b). This plant produces a yellow fruit with one to three seeds, covered by a white pulp. The fruit is used as food and possesses a characteristic acid flavor (Lim, 2012b). Chemical and biological studies for this species are scarce. In 2006, Suffredini et al., evaluated the antibacterial activity of the organic extracts (methanol:CH_2Cl_2 1:1) obtained from leaves and stems of *G. madruno*, a significant activity against *S. aureus* was found. Osorio et al. (2009) reported the antioxidant activity of a biflavonoid-enriched fraction (morelloflavone, volkensiflavone and amentoflavone) obtained from leaves and twigs of *G. madruno*. In 2013; Osorio et al., identified five known biflavonoids (morelloflavone, volkensiflavone, amentoflavone, fukugiside, madrunoudeaside and spicataside) and a new biflavonoid (7″-O-(6‴-acetyl)-glucoside of morelloflavone) from the aerial parts of *G. madruno*. In the same study, the antioxidant potential of the isolated metabolites was also evaluated by means of TBARS assay. More recently, Carrillo-Hormaza et al. (2016), reported a complete profile of biflavonoids from *G. madruno* and their antioxidant activities.

Taking into account the limited number of studies with this plant, the importance of the genus *Garcinia* as a source of bioactive metabolites; as well as previous studies carried out with different parts from other species of this genus (Hemshekhar et al., 2011), here we describe a) the quantification of garcinol, morelloflavone and volkensiflavone in fractions obtained mainly from leaves, seeds and epicarp of *G. madruno*, and b) the evaluation of the antioxidant activity of the extracts and compounds obtained from this tropical species.

2. Materials and methods

2.1. Reagents

Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), [2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] (ABTS) and 2,4,6-tripyridyl-*s*-trazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA); Na_2CO_3, NaOH, gallic acid, ascorbic acid, potassium persulphate, fluorescein, $FeCl_3$, ethanol, and HCl were acquired from Alfa-Aesar (Ward Hill, MA, USA). [2,2′-Azobis (2-amidinopropane) dihydrochloride] (AAPH) was purchased from Wako Pure Chemical USA (Richmond, VA, USA). All chemicals and reagents used were of analytical grade.

2.2. General methods

UV spectra were recorded on a Spectronic Genesys 5. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD on a Bruker AMX 300 spectrometer (^1H at 300 MHz; ^{13}C 75.5 MHz). 1D and 2D experiments (DEPT, ^1H-^1H COSY, HMQC and HMBC) were carried out for structure determinations. FTIR spectra were measured using $CHCl_3$ on a Perkin-Elmer RXI. Thin layer chromatography (TLC) was performed in silica gel F_{254} (0.2 mm Merck) plates; compounds were detected by UV fluorescence and/or spraying with H_2O-H_2SO_4-AcOH (30:28:143, v/v), followed by heating at 80 °C for 1–2 min. Column chromatography (CC) was carried out using silica gel or Sephadex LH-20 as stationary phases. High performance liquid chromatography (HPLC-DAD) was carried out using a Shimadzu Prominence HPLC system (Shimadzu, Kyoto, Japan), equipped with auto sampler, degasser, reciprocating pumps, diode array detector and the software LC solution 1.22 SP1.

2.3. Plant material

Ripened fruits of *G. madruno* were purchased from local market in Medellín, Colombia. Leaves were collected in the campus of the Universidad Nacional de Colombia-Sede Medellín. The specimen was identified by specialist and a voucher specimen (MEDEL-66811) was deposited at the Herbarium "Gabriel Gutiérrez Villegas" of the Universidad Nacional de Colombia-Sede Medellín for future reference.

2.4. Plant material extraction

The ripe fruits were washed thoroughly, epicarp (411.9 g) and seeds (735.9 g) were separated, shade dried and powdered using blender. In the same way, leaves (2.62 g) of *G. madruno* were powdered. Then, a weighed amount of the powder was exhaustively and successively extracted by percolation using *n*-hexane (H), dichloromethane (D), ethyl acetate (A) and methanol (M). Extracts were filtered, concentrated, under reduced pressure at 40 °C using a rotary evaporator (Buchi R-200), and used for the determination of total phenolic content, radical scavenging and antioxidant activities.

2.5. Total phenolic content determination

Total phenolic content was determined according to the Folin-Ciocalteau colorimetric method using gallic acid as a standard phenolic compound. The assay was performed as described by Zamorano et al. (2017) (for a more detailed description of method see supplementary material section).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jksus.2018.07.017.

2.6. Antioxidant activity determinations

The antioxidant capacity of the plant extracts and pure compounds from *G. madruno* were simultaneously assessed using the four following assay: (1) DPPH free radical scavenging activity, (2) ABTS radical cation scavenging activity, (3) Ferric reducing power assay (FRAP), and (4) Hydrophilic oxygen radical absorbance capacity assay (H-ORAC). The assays were performed as described by Zamorano et al. (2017). All antioxidant assays were conducted in triplicate (for a more detailed description of methods see supplementary material section).

2.7. Isolation of metabolites

The *n*-hexane, dichloromethane and ethyl acetate extracts from seeds of *G. madruno* were separately subjected to column chromatography and eluted with solvent mixture (*n*-hexane and ethyl acetate) of increasing polarity. The fractions were combined according to TLC profile, further chromatographed on silica gel CC and eluted with various mixtures of *n*-hexane and ethyl acetate. Finally, subfractions were separated by size-exclusion CC on the lipophilic organic resin Sephadex LH 20® column, and eluted with *n*-hexane, dichloromethane, methanol, 2:1:1 (v/v) to yield four pure compounds.

In order to determine the active compounds, **H**, **D** and **A** fractions from seeds of *G. madruno* were submitted to successive column chromatography (CC) using silica gel and Sephadex LH-20. Fractions **H** and **D** were combined and the resulting extract was submitted to CC on silica, with elution using *n*-hexane/EtOAc mixtures. A white solid was found in fractions 8 to 10, eluted with *n*-hexane. In turn, sub-fractions 29–31 eluted with *n*-hexane-ethyl acetate (70:30) were combined and rechromatographed by CC on Sephadex LH-20 using *n*-hexane-dichloromethane-methanol, 50:25:25, to afford a pure compound (pale yellow needle crystals). Both metabolites were characterized as glyceryl palmitate and garcinol by spectroscopic methods and comparison with literature data (Gunstone, 1991; Rao et al., 1980).

Fraction **A**, from seeds of *G. madruno*, was submitted to silica CC using as mobile phase *n*-hexane-ethyl acetate (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, and 0:100). Further, the last sub-fraction was submitted to silica CC to afford ten fractions. Sub-fractions 11–14 (eluted with *n*-hexane-ethyl acetate, 80:20) and 19-25 (eluted with *n*-hexane-ethyl acetate, 40:60) were separately combined and subjected to CC on Sephadex LH-20 using *n*-hexane-dichloro methane-methanol, 50:25:25, affording two pure compounds. Both compounds were characterized as morelloflavone and volkensiflavone by spectroscopic methods and comparison with literature data (Osorio et al., 2009; Duddeck et al., 1978; Chari et al., 1977) (see supplementary material).

2.8. Quantification of isolated metabolites

Reverse phase chromatographic analyses were performed under gradient conditions using a Phenomenex Security Guard cartridge C18 (4.0 × 3.0 mm) followed by a Zorbax Eclipse Plus C18 column (4.6 mm × 150 mm, 5 μm diameter particles, Agilent, USA). The compounds were eluted at a flow rate of 1.0 mL/min with the solvents A = acetic acid (1%) in water, and B = acetonitrile. Biflavonoid were eluded as follows: starting conditions of 10% of B were used; the % of solvent B increased to 35% over the next 11 min; then further increased to 70% over the next 9 min and held for 5 min. Under these conditions retention time were 17.9 and 18.1 min for morelloflavone and volkensinflavone, respectively. For garcinol separation, the gradient elution was performed with a linear increased of B from 50% to 100% in 20 min, and finally holding for 10 min 100% B. Injection volume was 20 μL. Compounds were monitored at 254 nm, although diode-array detection was used over a wavelength range of 200–800 nm to collect spectral data. Quantification of metabolites was carried out using standard calibration curves (peak areas vs. compound concentration for different concentrations). Six working solutions were prepared for each standard in methanol containing each pure compound between 5 and 200 μg/mL. All calibration curves presented high linearity ($R^2 > 0.96$). Data for each peak were collected using the wavelength of maximum response. The results were expressed as mg of compound per gram of extract (% w/w dry weight).

Table 1
Yield of sequential extraction of *G. madruno* with different solvents.

Extracts	Yield (mg/g)		
	Seeds	Epicarp	Leaves
Hexane (**H** fraction)	36.46	36.78	33.73
Dichloromethane (**D** fraction)	7.17	14.39	12.49
Ethyl acetate (**A** fraction)	5.44	8.74	21.70
Methanol (**M** fraction)	13.57	33.86	145.27

2.9. Statistical analysis

Data are expressed as mean ± standard deviation (SD) and subjected to one-way analysis of variance (ANOVA). Fisher's least significant difference test (LSD) at the 95.0% confidence level was performed to compare the statistically significant differences between antioxidant activities of fractions.

3. Results and discussion

Sequential extracts of seeds, epicarp, and leaves of *G. madruno* with different solvents of increasing polarity were prepared. Table 1 gives the percentage yield during the sequential extraction with *n*-hexane, dichloromethane, ethyl acetate and methanol. Yields ranged from 5.44 to 36.46, 8.74 to 36.78, and 12.49 to 145.27 mg/g for seeds, epicarp, and leaves, respectively. In the case of seeds and epicarp, the maximum yields were shown in the **H** fraction followed by **M** fraction. In leaves, the maximum yield was obtained in the extraction with methanol followed by the extraction with hexane. The sequential extraction with the same material allows ensuring the complete extraction of a wide polarity range of compounds.

Next, total phenolic content (TPC) of each extract was estimated using the Folin-Ciocalteu method. In addition, *in vitro* antioxidant potential of each extract was analyzed using the following methods: DPPH radical scavenging, ABTS cationic radical scavenging and ferric reducing activity assays (FRAP). Results of total phenolic content, radical scavenging and antioxidant activities of the extracts from seeds, epicarp, and leaves of *G. madruno* are presented in Table 2.

It is well known that the antioxidant activity of the plant extracts usually appears to correlate with the TPC values (Miliauskas et al., 2004). Fractions for each organ showed statistical differences in the TPC values ranging from 2404 ± 121 mg GAE/100 g for **H** fraction to 9097 ± 275 mg GAE/100 g for **A** fraction from seeds, 2372 ± 153 mg GAE/100 g for **D** fraction to 11292 ± 962 mg GAE/100 g for **A** fraction for epicarp, and 3659 ± 180 mg GAE/100 g for **D** fraction to 23510 ± 255 mg GAE/100 g for **A** fraction from leaves of *G. madruno*. The highest TPC values were found in **A** fractions for each organ. By contrast, the lowest TPC values

Table 2
Total phenolic content (TPC) and antioxidant activity of extracts obtained with solvents of different polarity from *Garcinia madruno* seeds, epicarp, and leaves.

Organ	Extract	Total phenolic content (TPC) and antioxidant activity			
		TPC[≠]	DPPH[†]	ABTS[§]	FRAP[‡]
Seeds	**H**	2404 ± 121^{a}	14913 ± 1361^{a}	26495 ± 237^{b}	1456 ± 138^{c}
	D	4577 ± 375^{b}	29444 ± 2098^{c}	41133 ± 2365^{c}	1335 ± 36^{c}
	A	9097 ± 275^{c}	23527 ± 2170^{b}	57069 ± 1350^{d}	969 ± 31^{b}
	M	2504 ± 215^{a}	13410 ± 1647^{a}	17885 ± 510^{a}	498 ± 2^{a}
Epicarp	**H**	5756 ± 227^{c}	26814 ± 772^{d}	50920 ± 1433^{c}	1554 ± 118^{b}
	D	2372 ± 153^{a}	21253 ± 964^{b}	34226 ± 1707^{b}	1186 ± 26^{a}
	A	11292 ± 962^{d}	23468 ± 1232^{c}	67768 ± 2990^{d}	2942 ± 146^{c}
	M	3407 ± 42^{b}	15090 ± 1070^{a}	25826 ± 1253^{a}	1020 ± 42^{a}
Leaves	**H**	4614 ± 69^{b}	6993 ± 622^{a}	60638 ± 5245^{b}	4366 ± 175^{b}
	D	3659 ± 180^{a}	5170 ± 366^{a}	42550 ± 1574^{a}	2921 ± 56^{a}
	A	23510 ± 255^{c}	83052 ± 3385^{c}	377848 ± 6039^{d}	21448 ± 199^{d}
	M	12279 ± 161^{d}	45772 ± 1369^{b}	208003 ± 7016^{c}	10875 ± 115^{c}

Conventions: H, hexane fraction; **D**, dichloromethane fraction; **A**, ethyl acetate fraction; **M**, methanol fraction. [≠]mg equiv. gallic acid/100 g sample. [†,§] TEAC (μmol equiv. Trolox/100 g sample). [‡]mg equiv. ascorbic acid/100 g of sample. For each organ, different letters within the same column indicate significant difference by the Fisher's least significant difference test (at 95.0% confidence level).

were detected in **H** and **M** fraction for seeds, and **D** fraction for epicarp and leaves. Among the different organs, **A** fraction from leaves of *G. madruno* offered the highest TPC values, followed by epicarp. Similar results were found with *G. madruno* samples; where epicarps and leaves showed the highest TPC values (Carrillo-Hormaza et al., 2016). The high yields of **H** fraction in all organs but low TPC values may be attributable to the content of more non-phenolic compounds, such as waxes, terpenes or steroids. Similarly, **M** fractions in all materials exhibited high yields but low TPC values for seeds and epicarp. This may also be due to the content of polar non-phenolic compounds such as carbohydrates. On the contrary, **A** fractions in seeds and epicarp presented lowest yields but displayed highest TPC values. Based on the results of TPC, the best extracting solvent was ethyl acetate. Different *Garcinia* species have shown a similar trend; ethyl acetate fraction exhibited the highest TPC values (Marlin and Katrin 2017; See et al., 2017; Meng et al., 2012).

DPPH radical scavenging assay is known to be one of the various methods for measuring antioxidant activity. The DPPH assay provides information about the interaction of tested compounds with the free radicals and its effect is thought to be due to the capacity of compounds for donating radical hydrogen (Younes et al., 2015). DPPH radical scavenging activity of all fractions obtained of *G. madruno* is showed in Table 2. For seeds, the maximum DPPH radical scavenging activity was recorded in **D** fraction followed by **A** fraction. No significant differences in DPPH radical scavenging properties between **H** fraction and **M** fraction were noted. Correlation between TPC and DPPH assay for seeds was at the level of $R^2 = 0.328$. For epicarp, all fractions showed statistical differences; the highest DPPH radical scavenging activity was obtained in the **H** fraction, followed by the **A** and **D** fractions. The linear relationship between TPC and DPPH values was lower ($R^2 = 0.207$) than those found for seeds. Among fractions obtained from leaves of *G. madruno*, **A** fraction offered the highest DPPH radical scavenging activity followed by **M** fraction. The lowest DPPH radical scavenging activities were displayed by the fractions obtained by extraction with the solvents of less polarity, **H** and **D** fractions. A very high correlation between TPC and DPPH for leaves was found ($R^2 = 0.990$). Based on the results of DPPH radical scavenging activity, the best extracting solvents were dichloromethane, hexane and ethyl acetate for seeds, epicarp, and leaves, respectively.

ABTS cationic radical scavenging activity of the fractions of *G. madruno* extracted with solvents of different polarity is summarized in Table 2. Results show that fractions for each organ presented statistical differences in the ABTS radical scavenging properties. For all organs, **A** fraction exhibited the highest ABTS cationic radical scavenging properties. For seeds, antioxidant activity decreased in the following order: ethyl acetate > dichlorome thane > hexane > methanol. There was a good correlation between TPC and ABTS radical scavenging capacity was found ($R^2 = 0.902$). For epicarp, ABTS radical scavenging activity decreased in the order: ethyl acetate > hexane > dichloromethane > methanol. The linear relationship between TPC values and ABTS scavenging activity for epicarp was at the level of $R^2 = 0.553$. In addition, ABTS assay revealed a decreasing order of radical scavenging activity: ethyl acetate > methanol > hexane > dichloromethane, for leaves of *G. madruno*. Correlation between TPC and ABTS assay for leaves was at the level of $R^2 = 0.562$. Based on the results of ABTS radical scavenging activity, the best extracting solvent was ethyl acetate.

FRAP assay measures the reducing potential of an antioxidant agent reacting with a ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex and produces a blue-colored complex of ferrous-tripyridyltriazine (Fe^{2+}-TPTZ). The FRAP assay is widely used in the search of antioxidant compounds from vegetal extracts. For seeds, for fractions **H** and **D** significant differences were not found. Results show that the antioxidant activity decreased as the polarity of the extracting solvent increased. The highest antioxidant activities were displayed by fractions **H** and **D** which were obtained by extraction with the solvents of less polarity. The correlation between TPC and FRAP assay was at the level of $R^2 = 0.001$. Among the extracts from epicarp and leaves of *G. madruno*, **A** fraction offered the highest antioxidant activity. For epicarp, the antioxidant capacity was decreased according to the following order of extracting solvent: ethyl acetate > hexane > dichloromethane $\geq$ methanol. A good linear relationship between TPC and FRAP assay was found at the level of $R^2 = 0.802$. For leaves, the antioxidant capacity was decreased according to the order of extracting solvent: ethyl acetate > methanol > hexane > dichloromethane. The linear relationship between TPC and FRAP assay was lower ($R^2 = 0.615$) than those found for epicarp. In general terms, our results are consistent with previous studies carried out with *Garcinia benthamiana*, *Garcinia xanthochymus* and *Garcinia hombroniana*; where the best radical scavenging and antioxidant capacity was displayed by ethyl acetate fraction (Marlin and Katrin, 2017; See et al., 2017; Meng et al., 2012). Likewise, Carrillo-Hormaza et al. (2016) also described epicarp and leaves from *G. madruno* as the samples with the highest antioxidant values.

A total of four compounds were isolated from *G. madruno* through consecutive column chromatographic separations (Fig. 1). Benzophenones are metabolites commonly found in *Garcinia* genus (Kumar et al., 2013). Although Carrillo-Hormaza et al. in 2016, by means of HPLC-DAD-ESI-MS^n, reported the presence of peaks associated with polyisoprenylated benzophenones, no compounds of this type have been reported for *G. madruno*. Therefore, garcinol, a benzophenone also known as camboginol, which has been found on different *Garcinia* species (Aravind et al., 2016a,b), is isolated and characterized for the first time from this species. This compound has received a lot of attention due to its health beneficial effects, mostly attributed to their potent antioxidant activity (Tang et al., 2013).

Morelloflavone and volkensiflavone biflavonoids have been previously reported in *G. madruno* by Osorio et al. (2009); and are widely distributed in *Garcinia* species (e.g. *G. morella*, *G. brasiliensis*, *G. talboti*, *G. spicata*, *G. multiflora*, *G. linii*, among others) (Locksley, 1973). Morelloflavone exhibits a wide array of biological activities, such as antioxidant, antiviral, anticancer, anti-inflammatory, hypercholesterolemic, cardiovascular, among others (Lim, 2012a). On the other hand, volkensiflavone has been tested in different models of biological activity with variable results (Lin et al., 2001; Verdi et al., 2004; Brusotti et al., 2016).

Quantitative determinations of garcinol, morelloflavone, and volkensinflavone were carried out by HPLC analyses on a C18 reversed phase column and by the calibration curve method. The regression equations were: garcinol, $y = 80651x - 541076$ ($R^2 = 0.996$), detection limit = 15.0 mg/g; morelloflavone, $y = 163798x - 885611$ ($R^2 = 0.963$), detection limit = 5.2 mg/g; and volkensiflavone, $y = 92139x - 82667$ ($R^2 = 0.994$), detection limit = 5.6 mg/g (Fig. 2). The results of the quantification of compounds showed that the *n*-hexane soluble fraction contained the highest level of garcinol, while little amount was detected in CH_2Cl_2 and EtOAc-soluble fractions (Table 3). In the **H** fraction, a concentration of 308.5, 398.9 and 36.8 mg/g of garcinol was found in seeds, epicarp and leaves, respectively. Morelloflavone and volkensiflavone were almost absent in **H** and **D** fractions, and only undetectable concentrations (<detection limit) were determined in each evaluated tissue. Among the biflavonoids, volkensiflavone was the major compound. For **A** and **M** fractions, concentrations of volkensiflavone of 203.8 and 93.6, 315.7 and 94.2, 175.6 and 63.2 mg/g extract were established in that order for seeds, epicarp, and leaves. Meanwhile, morelloflavone was found for **A** and **M** fractions at concentrations of 59.9 and 8.1, 6.5 and 7.1, and 104.0 and 52.6 mg/g extract for seeds, epicarp, and leaves, respectively. As previously reported, biflavonoids

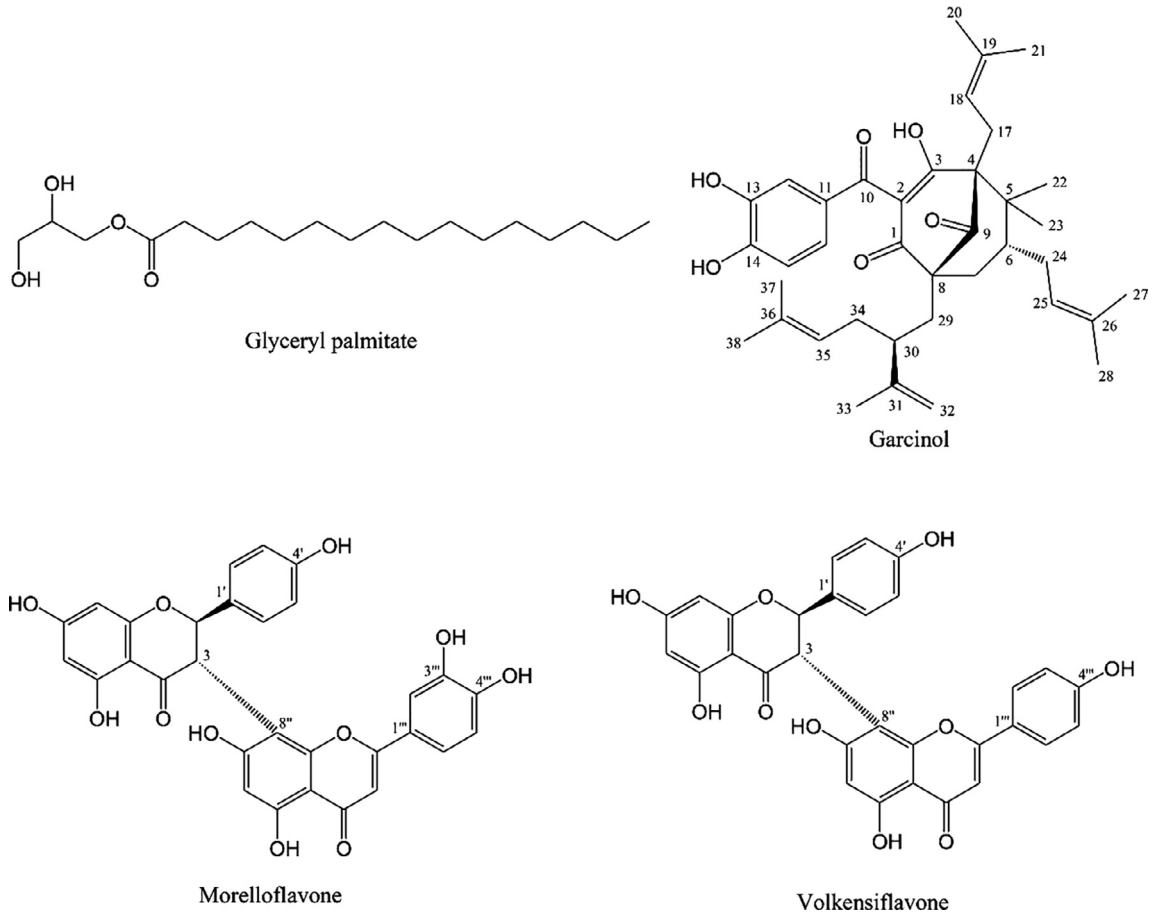

Fig. 1. Structure of compounds isolated from *G. madruno*.

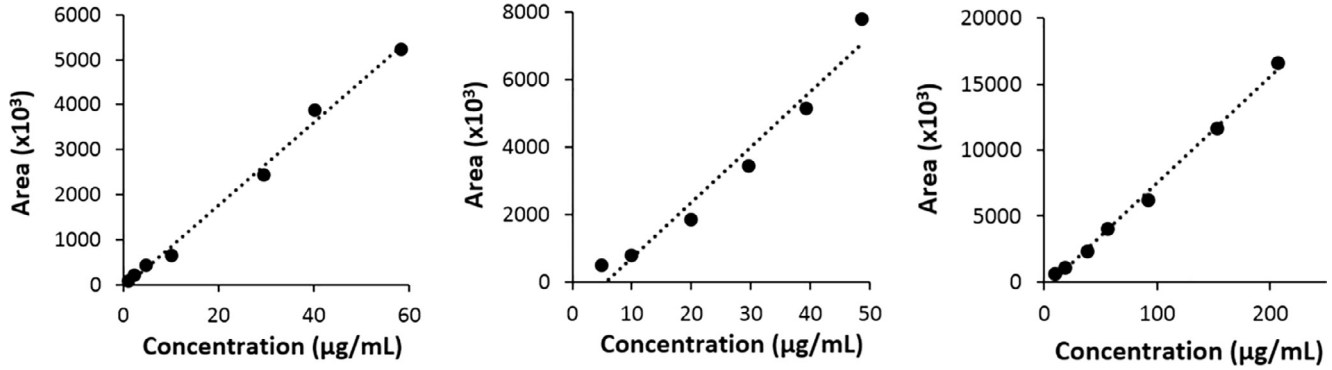

Fig. 2. Calibration curve of morelloflavone (left), volkensiflavone (center), garcinol (right).

Table 3
Quantification of isolated compounds from *G. madruno*.

Compound	Concentration (mg/g extract)											
	Seeds				Epicarp				Leaves			
	H	D	A	M	H	D	A	M	H	D	A	M
Volkensinflavone	n.d	n.d	203,8	93,6	n.d	n.d	315,7	94,2	n.d	n.d	175,6	63,2
Morelloflavone	n.d	n.d	59,9	8,1	n.d	n.d	6,5	7,1	n.d	n.d	104,0	52,6
Garcinol	308,5	41,3	32,3	n.d	398,9	171,0	30,4	n.d	36,8	n.d	n.d	n.d

Conventions: **H**, hexane fraction; **D**, dichloromethane fraction; **A**, ethyl acetate fraction; **M**, methanol fraction; **n.d.** not detected.

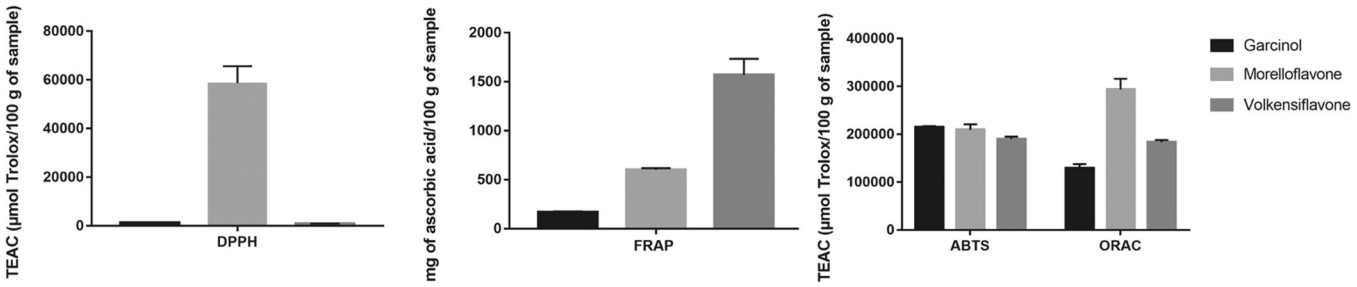

Fig. 3. Free radical scavenging and antioxidant activity of isolated compounds from *G. madruno*.

volkensiflavone and morelloflavone were found in all samples analysed (seeds, epicarp and leaves). In that study authors found morelloflavone as the main biflavonoid in epicarp and leaves; different to our results where volkensiflavone was the major compound (Carrillo-Hormaza et al., 2016). Differences in content could be associated with extraction methods employed, collection time, and sample origin, among others.

According to results, the highest amount of volkensiflavone was detected in the EtOAc-soluble fractions. In addition, the epicarp was found to be the tissue with the highest content of volkensiflavone, followed by the seeds. The **A** fractions from leaves of *G. madruno* showed the greatest amount of morelloflavone, followed by seeds. The amount of garcinol was decreased in the order epicarp, seeds, and leaves. In general, it can be seen that *G. madruno* accumulate high levels of these three compounds. These results are in agreement with previous studies that report the concomitant presence of benzophenones and biflavonoids in *Garcinia* genus (Aravind et al., 2016a,b).

Additionally, garcinol, morelloflavone and volkensiflavone were evaluated for their radical scavenging and antioxidant activity by DPPH, ABTS, FRAP, and ORAC assays. The antioxidant properties of pure compounds are summarised in Fig. 3. Garcinol, a polyisoprenylated benzophenone isolated from *n*-hexane and dichloromethane extracts, possess a moderate antioxidant effect with values of 1331 ± 73, 214433 ± 2657 and 129129 ± 8433 µmol Trolox/100 g of sample for the DPPH, ABTS and ORAC assays, respectively, and 168 ± 8 mg of ascorbic acid/100 of g sample for the FRAP assay. For this compound, various authors have reported significant antioxidant activity using different methods (Messi et al., 2014; Stark et al., 2015b).

The antioxidant activity of garcinol with stable free radical DPPH has been related with the presence of phenolic hydroxyl groups, the β-diketone moiety and the double bond of the isoprenyl group (Sang et al. 2002, 2001). Garcinol showed nearly three times higher DPPH free radical scavenging activity than DL-α-tocopherol by weight (Yamaguchi et al., 2000).

Among the biflavonoids, morelloflavone exhibited the strongest radical scavenging activity according to the DPPH, ABTS and ORAC assays with values of 58697 ± 6944, 209216 ± 11723, and 293842 ± 22026 µmol Trolox/100 g of sample, respectively. On the contrary, volkensiflavone displayed the highest antioxidant activity on the FRAP assay. Our results are in accordance with Panthong et al., 2009, who isolated morelloflavone and volkensiflavone with other three compounds from an acetone extract of *Garcinia cowa*. They reported that only morelloflavone showed a high antioxidant activity against DPPH. Additionally, Muriithi et al. (2016) and Carrillo-Hormaza et al. (2016) obtained similar results. In their studies, morelloflavone showed a higher scavenging activity as compared to volkensiflavone in DPPH and ORAC assays. These results were opposite to the results obtained by Stark et al. (2015a) where volkensiflavone showed higher activity than morelloflavone in ABTS and ORAC models of antioxidant activity.

Structurally, morelloflavone is a biflavonoid comprising a flavanone, naringenin covalently linked to a flavone, luteolin (3-8″-naringenilluteolin). Volkensiflavone consists of naringenin and apigenin (3-8″-naringenilapigenin). The high capacity of flavonoids to scavenge free radicals ($R^{\cdot}$) *in vitro* may be explained by their ability to donate hydrogen radicals ($H^{\cdot}$) from the hydroxyl groups. This reaction gives the flavonoid phenoxyl radicals and a stable molecule (RH). As expected from its phenolic nature, both bioflavonoids scavenge free radical. Nonetheless, the notable radical scavenging activity of morelloflavone has been related with the hydroxyl groups at the B ring; the *o*-dihydroxyl unit (*o*-catechol) present in the B ring is an important structural requirement for the formation of less reactive flavonoid phenoxyl radical compared to the free radical ($R^{\cdot}$) (Amić et al., 2003; Bors et al., 1990). Our results suggested that *G. madruno* might be a good source of metabolites with antioxidant activity and confirms the biflavone morelloflavone as a potent antioxidant metabolite (Gontijo et al., 2012).

4. Conclusions

G. madruno showed significant free radical scavenging and antioxidant activity in *n*-hexane, dichloromethane and ethyl acetate extracts. From bioactive fractions, four compounds were isolated, including the polyisoprenylated benzophenone garcinol, first time reported in this species, and the bioflavonoids morelloflavone and volkensiflavone. The three compounds displayed a significant free radical scavenging and antioxidant activity. *G. madruno* might be a good source of extracts or metabolites with a notable free radical scavenging and antioxidant activity.

Acknowledgements

The authors thank Universidad Nacional de Colombia-Sede Medellín for financial support and Prof. W. Quiñones (Universidad de Antioquia) for all NMR measurements.

Conflict of interest

The authors declare no conflict of interests publishing this article in this journal.

Authors' contribution

CR contributed in carrying out the laboratory work, plant material extraction and compound isolation. BR conducted the antioxidant assays. JHG and JCML performed the HPLC analysis, interpretation of spectroscopic data, and contributed to the structuring and critical reading of the manuscript. DLD designed the study, supervised the laboratory work and revised the final version of the paper. All the authors have read the final manuscript and approved the submission.

References

Amić, D., Davidović-Amić, D., Bešlo, D., Trinajstić, N., 2003. Structure-radical scavenging activity relationships of flavonoids. Croat. Chem. Acta 76 (1), 55–61.

Aravind, A.P.A., Asha, K.R.T., Rameshkumar, K.B., 2016a. Phytochemical analysis and antioxidant potential of the leaves of *Garcinia travancorica* Bedd. Nat. Prod. Res. 30 (2), 232–236.

Aravind, A.P.A., Menon, A.L.N., Rameshkumar, K.B., 2016b. Structural diversity of secondary metabolites in Garcinia species. In: Rameshkumar, K.B. (Ed.), Diversity of Garcinia species in the Western Ghats: Phytochemical Perspective. Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Kerala, India, pp. 19–75.

Bors, W., Heller, W., Michel, C., Saran, M., 1990. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. Methods Enzymol. 186, 343–355.

Brusotti, G., Papetti, A., Serra, M., Temporini, C., Marini, E., Orlandini, S., Sanda, A.K., Watcho, P., Kamtchouing, P., 2016. *Allanblackia floribunda* Oliver: an aphrodisiac plant with vasorelaxant properties. J. Ethnopharmacol. 192, 480–485.

Carrillo-Hormaza, L., Ramírez, A.M., Quintero-Ortiz, C., Cossio, M., Medina, S., Ferreres, F., Gil-Izquierdo, A., Osorio, E., 2016. Comprehensive characterization and antioxidant activities of the main biflavonoids of *Garcinia madruno*: a novel tropical species for developing functional products. J. Funct. Foods 27, 503–516.

Chari, V.M., Ilyas, M., Wagner, H., Neszmélyi, A., Fa-Ching, C., Li-Kuang, C., Yu-Chin, L., Yu-Meei, L., 1977. 13C-NMR spectroscopy of biflavanoids. Phytochemistry 16 (8), 1273–1278.

Dal Molin, M.M., Silva, S., Alves, D.R., Quintão, N.L., Delle Monache, F., Cechinel Filho, V., Niero, R., 2012. Phytochemical analysis and antinociceptive properties of the seeds of *Garcinia achachairu*. Arch. Pharm. Res. 35 (4), 623–631.

Duddeck, H., Snatzke, G., Yemul, S.S., 1978. 13C NMR and CD of some 3,8″-biflavanoids from Garcinia species and of related flavanones. Phytochemistry 17 (8), 1369–1373.

Elfit, E., Muharni, M., Latief, M., Darwati, D., Widiyantoro, A., Supriyatna, S., Bahti, H. H., Dachriyanus, D., Cos, P., Maes, L., Foubert, K., Apers, S., Pieters, L., 2009. Antiplasmodial and other constituents from four Indonesian *Garcinia* spp. Phytochemistry 70 (7), 907–912.

Gontijo, V.S., Corrêa de Souza, T., Aparecido Rosa, I., Gomes Soares, M., Aparecido da Silva, M., Vilegas, W., Viegas Júnior, C., Henrique dos Santos, M., 2012. Isolation and evaluation of the antioxidant activity of phenolic constituents of the *Garcinia brasiliensis* epicarp. Food Chem. 132 (3), 1230–1235.

Gunstone, F.D., 1991. 13C-NMR studies of mono-, di-and tri-acylglycerols leading to qualitative and semiquantitative information about mixtures of these glycerol esters. Chem. Phys. Lipids. 58 (3), 219–224.

Hemshekhar, M., Sunitha, K., Sebastin Santhosh, M., Devaraja, S., Kemparaju, K., Vishwanath, B.S., Niranjana, S.R., Girish, K.S., 2011. An overview on genus *Garcinia*: phytochemical and therapeutical aspects. Phytochem. Rev. 10 (3), 325–351.

Kumar, S., Sharma, S., Chattopadhyay, S.K., 2013. The potential health benefit of polyisoprenylated benzophenones from *Garcinia* and related genera: Ethnobotanical and therapeutic importance. Fitoterapia 89 (1), 86–125.

Lim, T.K., 2012a. *Garcinia dulcis*. In: Lim, T.K. (Ed.), Edible Medicinal and Non-Medicinal Plants Volume 2, Fruits. Springer, Netherlands, pp. 35–39.

Lim, T.K., 2012b. *Garcinia madruno*. In: Lim, T.K. (Ed.), Edible Medicinal and Non-Medicinal Fruits Volume 2, Plants. Springer, Netherlands, pp. 76–79.

Lin, Y.M., Flavin, M.T., Cassidy, C.S., Mar, A., Chen, F.C., 2001. Biflavonoids as novel antituberculosis agents. Bioorg. Med. Chem. Lett. 11 (16), 2101–2104.

Lin, K.W., Huang, A.M., Yang, S.C., Weng, J.R., Hour, T.C., Pu, Y.S., Lin, C.N., 2012. Cytotoxic and antioxidant constituents from *Garcinia subelliptica*. Food Chem. 135 (2), 851–859.

Locksley, H.D., 1973. The chemistry of biflavanoid compounds. In: Grisebach, H., Kirby, G.W., Herz, W. (Eds.), Fortschritte Der Chemie Organischer Naturstoffe (Progress in the Chemistry of Organic Natural Products). Springer, Vienna, pp. 207–312.

Mackeen, M.M., Mooi, L.Y., Amran, M., Mat, N., Lajis, N.H., Ali, A.M., 2012. Noncytotoxic and antitumour-promoting activities of Garcinia acid esters from Garcinia atroviridis Griff. ex T. Anders (Guttiferae). Evid. Based. Complement. Alternat. Med. 829814.

Marlin, S., Katrin, E.B., 2017. Antioxidant Activity and Lipooxygenase Enzyme Inhibition Assay with Total Flavonoid Content from *Garcinia hombroniana* Pierre Leaves. Pharmacogn. J. 9 (2), 267–272.

Meng, F., Hui-Jin, F., Yu, C., De-Bin, W., Guang-Zhong, Y., 2012. Antioxidant activity of *Garcinia xanthochymus* leaf, root and fruit extracts *in vitro*. Chin. J. Nat. Med. 10 (2), 0129–0134.

Messi, B.B., Ho, R., Lannang, A.M., Cressend, D., Perron, K., Nkengfack, A.E., Carrupt, P., Hostettmann, K., Cuendet, M., 2014. Isolation and biological activity of compounds from *Garcinia preussii*. Pharm. Biol. 52 (6), 706–711.

Miliauskas, G., Venskutonis, P.R., van Beek, T.A., 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 85 (2), 231–237.

Muriithi, E., Bojase-Moleta, G., Majinda, R.R.T., 2016. Benzophenone derivatives from *Garcinia livingstonei* and their antioxidant activities. Phytochem. Lett. 18, 29–34.

Osman, M., Rahman, M.A., 2006. Mangosteen: *Garcinia mangostana*. Southampton Centre for Underutilised Crops, University of Southampton, Southampton, UK.

Osorio, D.E., Montoya, P.G., Bastida, J., 2009. Phytochemical characterization of a biflavonoid fraction from *Garcinia madruno*: inhibition of human LDL oxidation and its free radical scavenging mechanism. Vitae 16 (3), 369–377.

Osorio, E., Londoño, J., Bastida, J., 2013. Low-density lipoprotein (LDL)-antioxidant biflavonoids from *Garcinia madruno*. Molecules 18 (5), 6092–6100.

Otuki, M.F., Bernardi, C.A., Prudente, A.S., Laskoski, K., Gomig, F., Horinouchi, C.D., Guimarães, C.L., Ferreira, J., Delle-Monache, F., Cechinel-Filho, V., Cabrini, D.A., 2011. *Garcinia gardneriana* (Planchon & Triana) Zappi. (Clusiaceae) as a topical anti-inflammatory alternative for cutaneous inflammation. Basic Clin. Pharmacol. Toxicol. 109 (1), 56–62.

Panthong, K., Hutadilok-Towatana, N., Panthong, A., 2009. Cowaxanthone F, a new tetraoxygenated xanthone, and other anti-inflammatory and antioxidant compounds from *Garcinia cowa*. Can. J. Chem. 87 (11), 1636–1640.

Rao, A.V.R., Venkatswamy, G., Pendse, A.D., 1980. Camboginol and cambogin. Tetrahedron Lett. 21 (20), 1975–1978.

Sang, S., Liao, C.H., Pan, M.H., Rosen, R.T., Lin-Shiau, S.Y., Lin, J.K., Ho, C.T., 2002. Chemical studies on antioxidant mechanism of garcinol: analysis of radical reaction products of garcinol with peroxyl radicals and their antitumor activities. Tetrahedron 58 (51), 10095–10102.

Sang, S., Pan, M.H., Cheng, X., Bai, N., Stark, R.E., Rosen, R.T., Lin-Shiau, S.Y., Lin, J.K., Ho, C.T., 2001. Chemical studies on antioxidant mechanism of garcinol: analysis of radical reaction products of garcinol and their antitumor activities. Tetrahedron 57 (50), 9931–9938.

See, I., Cheng-Lian Ee, G., Hui-Mah, S., Mian-Jong, V.Y., Sin-Teh, S., 2017. Effect of Solvents on Phytochemical Concentrations and antioxidant activity of *Garcinia benthamiana* stem bark extracts. J. Herbs Spices Med. Plants 23 (2), 117–127.

Siridechakorn, I., Phakhodee, W., Ritthiwigrom, T., Promgool, T., Deachathai, S., Cheenpracha, S., Prawat, U., Laphookhieo, S., 2012. Antibacterial dihydrobenzopyran and xanthone derivatives from *Garcinia cowa* stem barks. Fitoterapia 83 (8), 1430–1434.

Stark, T.D., Lösch, S., Wakamatsu, J., Balemba, O.B., Frank, O., Hofmann, T., 2015a. UPLC-ESI-TOF MS-Based Metabolite Profiling of the Antioxidative Food Supplement *Garcinia buchananii*. J. Agric. Food. Chem. 63 (32), 7169–7179.

Stark, T.D., Salger, M., Frank, O., Balemba, O.B., Wakamatsu, J., Hofmann, T., 2015b. Antioxidative compounds from *Garcinia buchananii* stem bark. J. Nat. Prod. 78 (2), 234–240.

Suffredini, I.B., Paciencia, M.L.B., Nepomuceno, D.C., Younes, R.N., Varella, A.D., 2006. Antibacterial and cytotoxic activity of Brazilian plant extracts–Clusiaceae. Mem. Inst. Oswaldo Cruz 101 (3), 287–290.

Sweeney, P.W., 2008. Phylogeny and floral diversity in the genus *Garcinia* (Clusiaceae) and relatives. Int. J. Plant Sci. 169 (9), 1288–1303.

Tang, W., Pan, M-H., Sang, S., Li, S., Ho, C-T., 2013. Garcinol from Garcinia indica: Chemistry and Health Beneficial Effects. In: Tropical and Subtropical Fruits: Flavors, Color, and Health Benefits. Chapter 8, Vol. 1129, ACS Symposium Series, USA, pp. 133–145.

Verdi, L.G., Pizzolatti, M.G., Montanher, A.B.P., Brighente, I.M.C., Junior, A.S., E. de Smania F.A., Simionatto, E.L., Delle-Monache, F., 2004. Antibacterial and brine shrimp lethality tests of biflavonoids and derivatives of Rheedia gardneriana. Fitoterapia 75 (3–4), 360–363.

Xia, Z.X., Zhang, D.D., Liang, S., Lao, Y.Z., Zhang, H., Tan, H.S., Chen, S.L., Wang, X.H., Xu, H.X., 2012. Bioassay-guided isolation of prenylated xanthones and polycyclic acylphloroglucinols from the leaves of *Garcinia nujiangensis*. J. Nat. Prod. 75 (8), 1459–1464.

Yamaguchi, F., Ariga, T., Yoshimura, Y., Nakazawa, H., 2000. Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. J Agric. Food Chem. 48 (2), 180–185.

Younes, K., Merghache, S., Djabou, N., Selles, C., Muselli, A., Tabti, B., Costa, J., 2015. Chemical composition and free radical scavenging activity of essential oils and extracts of Algerian *Cardaria draba* (L.). Desv. J. Essen. Oil Bear. Pl. 18 (6), 1448–1458.

Zamorano, P., Rojano, B., Morales, M., Magariños, H., Godoy, P., Muñoz, O., 2017. Biological and antioxidant activity of *Gunnera tinctoria* (Nalca). J. Med. Plants Res. 11 (17), 318–330.