

## Research Article

# Determination of Polar Compounds in Guava Leaves Infusions and Ultrasound Aqueous Extract by HPLC-ESI-MS

Elixabet Díaz-de-Cerio,<sup>1,2</sup> Vito Verardo,<sup>3,4</sup> Ana María Gómez-Caravaca,<sup>1,2</sup>  
Alberto Fernández-Gutiérrez,<sup>1,2</sup> and Antonio Segura-Carretero<sup>1,2</sup>

<sup>1</sup>Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avenida Fuentenueva s/n, 18071 Granada, Spain

<sup>2</sup>Functional Food Research and Development Center, Health Science Technological Park, Avenida del Conocimiento, Bioregion Building, 18100 Granada, Spain

<sup>3</sup>Department of Chemistry and Physics (Analytical Chemistry Area), University of Almería, Carretera de Sacramento s/n, 04120 Almería, Spain

<sup>4</sup>Research Centre for Agricultural and Food Biotechnology (BITAL), Agrifood Campus of International Excellence, ceiA3, Carretera de Sacramento s/n, 04120 Almería, Spain

Correspondence should be addressed to Vito Verardo; [vito.verardo@unibo.it](mailto:vito.verardo@unibo.it)

Received 10 December 2014; Revised 4 March 2015; Accepted 7 March 2015

Academic Editor: Serkos A. Haroutounian

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Literature lacks publications about polar compounds content in infusion or guava leaves tea. Because of that, a comparison between different times of infusion and a conventional ultrasound aqueous extract was carried out. Several polar compounds have been identified by HPLC-ESI-MS and their antioxidant activity was evaluated by FRAP and ABTS assays. Four different classes of phenolic compounds (gallic and ellagic acid derivatives, flavonols, flavanones, and flavan-3-ols) and some benzophenones were determined. The quantification results reported that the order, in terms of concentration of the classes of polar compounds in all samples, was flavonols > flavan-3-ols > gallic and ellagic acid derivatives > benzophenones > flavanones. As expected, the aqueous extract obtained by sonication showed the highest content in the compounds studied. Significant differences were noticed about the different times of infusion and five minutes was the optimal time to obtain the highest content in polar compounds using this culinary method. All the identified compounds, except HHDP isomers and naringenin, were positively correlated with antioxidant activity.

## 1. Introduction

The studies on antioxidant activity of plants have increased dramatically in recent years, because they are identified as natural antioxidant resources by traditional Chinese medicine [1]. Medicinal plants have usually been applied to control the blood glucose or reduce the diabetic complications; they have the potential to increase the life span and quality of life in these patients [2]. The increasing prevalence of type 2 diabetes mellitus and the negative clinical outcomes observed with the commercially available antidiabetic drugs have led to the investigation of new therapeutic approaches focused on controlling postprandial glucose levels. The use of carbohydrate digestive enzyme inhibitors from natural

resources could be a possible strategy to block dietary carbohydrate absorption with less adverse effects than synthetic drugs. In fact, some authors [3] reported *in vitro* and *in vivo* studies in relation to pancreatic alpha-amylase inhibitors of plant origin and presented bioactive compounds of phenolic nature that exhibit anti-amylase activity.

Guava leaves (*Psidium guajava* L.) are considered native to Mexico but today they are extended throughout South America, Europe, Africa, and Asia. Different studies considered these leaves as a promising source of phenolic compounds for diabetes treatment [4]. Several authors noticed that oral administration of capsules containing aqueous leaf extract from *Psidium guajava* L. showed hypoglycemic effect [5].

Recently, Eidenberger and coworkers [6] investigated the effect of extracts from *Psidium guajava* L. leaves, particularly, the effects of main flavonol-glycoside components on dipeptidyl-peptidase IV (DP-IV), a key enzyme of blood glucose homeostasis, and, finally, indicated that guava extract has a potential to exert the effect observed *in vitro* also in humans after oral administration.

*In vivo* experiments carried out by Cheng et al. [7] reported that quercetin in the aqueous extract of guava leaves promotes glucose uptake in liver cells and as a consequence contributes to the alleviation of hypoglycemia in diabetes.

Usually, guava leaf tea was consumed after infusion; however, different infusion times were advice from production company. Because of that, in the present work, the antioxidant activities of infusions obtained at different infusion times and conventional ultrasound aqueous extracts of guava leaves were evaluated and compared in terms of their composition in polar compounds.

## 2. Material and Methods

**2.1. Chemicals.** Double-deionised water with conductivity lower than 18.2 M $\Omega$  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 and the standards gallic acid, catechin, ellagic acid, naringenin, quercetin, and rutin were all from Sigma-Aldrich (Steinheim, Germany). The reagents used to measure the antioxidant capacity, 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-sulfonate)), potassium persulfate, and ferric sulfate, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium acetate, ferric chloride, and hydrochloric acid were obtained from Panreac (Barcelona, Spain).

**2.2. Plant Material and Sample Preparation.** Fresh guava leaves were harvested in Motril, Spain (36°44'43"N, 3°31'14"O). They were middle age intense green leaves and they were collected in February 2014. The environmental conditions had mean max/min temperature of 18/10°C, precipitation of 0 mm, and saturated light duration that ranged from 9.55 to 10.50 h day<sup>-1</sup>.

The samples were air-dried and ground before the analyses. Two different extraction methodologies, such as ultrasound extraction and infusion, were carried out.

**Conventional Ultrasound Extraction.** 0.5 g of dry guava leaves was extracted with 15 mL of water ( $\times 3$ ) 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 50% methanol. This solution was filtered through a 0.20  $\mu$ m syringe filter and kept at -20°C in amber bottles to avoid degradation until analysis.

**Infusion Extraction.** For the infusion, 1 g of dried guava leaves and 50 mL of boiling water were used. The extract was

prepared according to the method previously described by Chen and Yen [8], where they prepared 5 min infusion and compared with infusion for 3 and 7 min. After the extraction by infusion for 3, 5, and 7 min, the solution was raised to 50 mL with water, filtered through a 0.20  $\mu$ m syringe filter, and kept at -20°C in amber bottles to avoid degradation until analysis.

All extractions were made in triplicate.

**2.3. Trolox Equivalent Antioxidant Capacity (ABTS) Assay.** The ABTS assay, which measures the reduction of the radical cation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) by antioxidants, was performed by using a method previously described by Laporta and coworkers [9]. Concisely, ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate in the dark at room temperature for 12–24 h before use. The absorbance of ABTS radical cation was adjusted to 0.70 ( $\pm 0.02$ ) at 734 nm. A calibration curve was prepared with different concentrations of Trolox (0–20  $\mu$ M).

**2.4. Ferric-Reducing Antioxidant Power (FRAP).** The reducing power was evaluated according to the method validated by Benzie and Strain [10]. Briefly, 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM aqueous FeCl<sub>3</sub> were prepared and mixed (10 : 1 : 1), to obtain the FRAP reagent. The FRAP reagent was warmed to 37°C, before reading its absorbance. Then, the samples were added. The change in absorbance (593 nm) between the samples and the blank was related to the absorbance of an aqueous solution of known Fe(II) concentration, prepared for calibration.

**2.5. HPLC-ESI-MS Analysis.** Phenolic and other polar compounds in the extracts obtained from guava leaves were identified using a method introduced by Chang et al. [11], with slight modifications. Briefly, HPLC analyses were performed using a HP 1100 Series instrument (Hewlett Packard, Wilmington, DE, USA) equipped with a binary pump delivery system, a degasser (model G1322A), an autosampler (Automatic Liquid Sampler, ALS, model G1312A), a HP diode-array UV-VIS detector (DAD, model G1315A), and a quadrupole HP-Mass Spectrometer Detector (MSD, model G1946A); integration and data elaboration were performed using Chemstation software (Hewlett Packard). A Phenomenex Luna C18 analytical column (150 mm  $\times$  2.0 mm, particle size 3  $\mu$ m) (Phenomenex Inc., Torrance, CA, USA) was used for polar compounds separation. All analyses were carried out at room temperature using the gradient proposed by Chang et al. [11]. MS analysis was carried out using an electrospray ionization (ESI) interface in negative ionization mode at the following conditions: drying gas flow (N<sub>2</sub>), 9.0 L/min; nebulizer pressure, 50 psi; gas drying temperature, 350°C; capillary voltage, 3500 V; and fragmentor voltage and scan range, 100 V and *m/z* 50–1000, respectively.

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 identified compounds were quantified on the basis of their peak area and compared with calibration curves obtained

with the corresponding standards and then expressed as  $\mu\text{g/g}$  of extract.

**2.6. Statistical Analysis.** The results reported in this study are the averages of three repetitions ( $n = 3$ ). Fisher's least significance difference (LSD) test and Pearson's linear correlations, both at  $P < 0.05$ , were evaluated using Statistica 6.0 (2001, StatSoft, Tulsa, OK).

### 3. Results and Discussion

**3.1. Identification of Polar Compounds.** Phenolic and other polar compounds were identified by their elution order, UV/vis spectra, and MS characteristics, compared with reported literature values, and by coinjection with available standards (Table 1).

About phenolic compounds, four different classes identified as gallic and ellagic acid derivatives, flavonols, flavanones, and flavan-3-ols were determined.

Thirteen gallic and ellagic acid derivatives were determined. Three compounds (**1**, **2**, and **3**) with molecular ion at  $m/z$  481 and fragment at  $m/z$  301 were identified as hexahydroxydiphenic acid (HHDP) glucose and its presence in guava was previously reported by Okuda and coworkers [12]. Gallic acid (compound **4**) was identified according to its MS data ( $m/z$  169 and  $m/z$  125) and by coelution with a chemical standard. Two compounds (**6** and **11**) with  $[\text{M}-\text{H}]^-$  at  $m/z$  783 and two fragments at  $m/z$  481 and 301 corresponding to loss of ellagic acid were detected. This fragmentation pattern was assigned to pedunculagin/casuarinin compounds; these compounds were described in guava leaves by Okuda et al. [12].

Two compounds (**10** and **12**) with molecular ion at  $m/z$  951 and fragments at  $m/z$  783 and 481 were also determined. These compounds were identified as geraniin isomers [13].

Two compounds (**15** and **18**) showing significant  $[\text{M}-\text{H}]^-$  signals at  $m/z$  785 with fragment ions at  $m/z$  615 and at  $m/z$  301 were found. This fragmentation pattern corresponded to a digalloyl-HHDP-glucose structure, probably tellimagrandin I isomer. This compound was previously detected in guava leaves by Okuda et al. [12]. Compound **17** at  $m/z$  935 reported a fragment ion at  $m/z$  783; this fragmentation pattern was assigned to casuarinin/casuarictin and this compound was described in guava tea by Yamanaka and coworkers [13]. Compound **30** was identified as ellagic acid due its coelution with commercial standard. Finally, guavin B (compound **46**) that reported a molecular ion at  $m/z$  693 was identified according to Okuda et al. [14].

Moreover, ten flavan-3-ol derivatives were determined. Compounds **5** and **42** showed a molecular ion at  $m/z$  609 and three fragments at  $m/z$  441, 423, and 305 (gallo catechin unit); these compounds were identified as prodelphinidin B2 and its isomer and their presence in guava leaves was noticed by Qa'Dan et al. [15].

Compounds **7** and **9** showed a molecular ion at  $m/z$  593 and two fragments at  $m/z$  425 and 407. According to Qa'Dan et al. [15], these compounds were identified as prodelphinidin dimer ( $4\alpha$ -8).

Two compounds (**8** and **19**) with  $[\text{M}-\text{H}]^-$  at  $m/z$  305 and fragment ions at  $m/z$  221 and 179 were identified as gallo catechin isomers and their presence in guava leaves was reported by Qa'Dan et al. [15]. Three procyanidin dimers ( $[\text{M}-\text{H}]^-$  at  $m/z$  577) were also described (compounds **13**, **14**, and **21**).

Catechin compound (**16**) was identified by mass spectra data elaboration and coelution with a commercial standard.

The flavonols were the most representative phenolic compounds; in fact, eighteen flavonol-derivatives were identified. Four compounds (compounds **20**, **24**, **27**, and **29**) reported a molecular ion at  $m/z$  449 and two major fragments at  $m/z$  316 and 317. According to Chang et al. [11], these compounds were identified as myricetin-arabinoside/xylopyranoside isomers.

Two flavonol compounds (compounds **22** and **23**) with molecular ion at  $m/z$  479 and fragments at  $m/z$  317 and 316 were identified as myricetin-hexoside isomers [11]. Two compounds (**25** and **26**) corresponding to  $[\text{M}-\text{H}]^-$  signals at  $m/z$  615 were also detected. Based on their molecular weight and the presence of two fragments at  $m/z$  463 and 301, they were assigned to quercetin-galloylhexoside isomers and their presence in guava leaves was reported by Park et al. [16].

Two compounds at  $m/z$  301 were detected (**28** and **47**); moreover, they showed the same fragment ion at  $m/z$  151. Quercetin standard solution was injected and because of that compound **28** was assigned to morin and compound **47** was assigned to quercetin; their presence in guava leaves was reported by several authors [11, 17].

Compounds detected at  $m/z$  463 (**31** and **33**) with fragment ion at  $m/z$  301 corresponded to hyperin and isoquercitrin, respectively. They have previously been found in leaves of guava by Eidenberger et al. [6].

Quercetin glucuronide (**32**) with molecular ion at  $m/z$  477 and fragment ions at  $m/z$  433 and 301 was identified according to Chang et al. [11].

Three compounds (**34**, **35**, and **37**) reported the same molecular ion ( $m/z$  433) and a fragment ion at  $m/z$  301 (corresponding to quercetin aglycone); according to their retention times, they were identified as reynoutrin, guajaverin, and avicularin as reported by Chang et al. [11].

Quercitrin (**38**) was identified at  $m/z$  447 and fragment ion  $m/z$  300, according to Park et al. [16].

A flavanone compound, namely, naringenin ( $m/z$  271), was detected and identified by analyzing the mass spectra and by coelution with a chemical standard [18]. Compound **39** with  $m/z$  585 was identified as guavinoside C [19].

Six benzophenone compounds were also determined in guava leaves sample. The  $[\text{M}-\text{H}]^-$  ion at  $m/z$  543 revealed the presence of two compounds, namely, guavinoside A isomers; their presence in guava leaves was noticed by Matsuzaki and coworkers [19].

Finally, four compounds with  $m/z$  571 (**40**, **43**, **44**, and **45**) were identified as guavinoside B isomers according to Matsuzaki et al. [19].

**3.2. Quantification of Polar Compounds.** Comparison between different times of infusion and a conventional ultrasound aqueous extract was carried out due to several

TABLE 1: Identification of polar compounds in guava leaves by HPLC-DAD-ESI-MS.

Number	Compounds	$\lambda_{\max}$ (nm)	$[M-H]^-$	Fragments
1	HHDP glucose isomer	290	481	301
2	HHDP glucose isomer	290	481	301
3	HHDP glucose isomer	290	481	301
4	Gallic acid	272, 225	169	125
5	Prodelphinidin B2 isomer	280, 360	609	423, 441, 305
6	Pedunculagin/casuariin isomer	253, 377	783	481, 301
7	Prodelphinidin dimer isomer	280, 340	593	407, 425
8	Gallocatechin	236, 270sh	305	179, 221
9	Prodelphinidin dimer isomer	280, 340	593	407, 425
10	Geraniin isomer	270	951	783, 481, 301
11	Pedunculagin/casuariin isomer	253, 377	783	481, 301
12	Geraniin isomer	270	951	783, 481
13	Procyanidin B isomer	278, 234	577	289
14	Procyanidin B isomer	278, 235	577	289
15	Tellimagrandin I isomer	279, 340	785	615, 301
16	Catechin	236, 281	289	245, 205, 179
17	Casuarinin/casuarictin isomer	238, 275sh	935	783
18	Tellimagrandin I isomer	277, 338	785	615, 301
19	Gallocatechin	236, 270sh	305	179, 221
20	Myricetin-arabinoside/xylopyranoside isomer	264, 231sh, 356	449	316, 317
21	Procyanidin B isomer 2	268, 350	577	425, 289
22	Myricetin hexoside isomer	261, 231sh, 358	479	316, 317
23	Myricetin hexoside isomer	264, 235sh, 356	479	316, 317
24	Myricetin-arabinoside/xylopyranoside isomer	264, 231sh, 356	449	316, 317
25	Quercetin-galloylhexoside isomer	268, 350	615	463, 301
26	Quercetin-galloylhexoside isomer	280, 345	615	463, 301
27	Myricetin-arabinoside/xylopyranoside isomer	256, 234sh, 356	449	316, 317
28	Morin	232sh, 257, 374	301	151
29	Myricetin-arabinoside/xylopyranoside isomer	257, 231sh, 356	449	316, 317
30	Ellagic acid	254, 370	301	257, 185
31	Hyperin	259, 355, 235sh	463	301
32	Quercetin glucuronide	265, 355, 233sh	477	301, 433
33	Isoquercitrin	258, 355, 235sh	463	301
34	Reynoutrin	258, 356, 231sh	433	301
35	Guajaverin	257, 356, 231sh	433	301
36	Guavinoside A	218, 288	543	—
37	Avicularin	257, 355, 231sh	433	301
38	Quercitrin	264, 353	447	300
39	Guavinoside C	211, 265, 355	585	—
40	Guavinoside B	218, 283	571	—
41	Guavinoside A isomer	218, 288	543	—
42	Prodelphinidin B2 isomer 2	282, 340	609	423, 441, 305
43	Guavinoside B isomer	218, 283	571	—
44	Guavinoside B isomer	218, 283	571	—
45	Guavinoside B isomer	218, 283	571	—
46	Guavin B	208, 221, 283	693	—
47	Quercetin	232sh, 257, 374	301	151
48	Naringenin	280	271	—

publications about phenolic and other polar compounds content in infusion or guava leaves tea.

Quantification of polar compounds was performed by preparing five calibration curves with the standards available: gallic acid, catechin, ellagic acid, naringenin, and rutin. For those with no commercial standard available, quantification was carried out comparing with compounds bearing similar structures.

It is important to underline that the quantification results reported that the order, in terms of concentration of the families of polar compounds in all samples, decreased in the following order: flavonols > flavan-3-ols > gallic and ellagic derivatives > benzophenones > flavanones.

In general, the results given in Table 2 show that the concentration of each compound is greater in the ultrasound aqueous extract (AE), except the compounds identified as HHDP glucose that was higher in the infusion of 3 min (I3) and in the 5 min (I5) samples and naringenin, which presented the largest concentration in I3. Similar results were obtained by Nantitanon and coworkers [20] using ethanol as extraction solvent. In fact, they extracted the guava leaves by maceration and ultrasounds, and the highest recovery of phenolic compounds was obtained by sonication.

The higher extraction of HHDP and naringenin in some infusions than ultrasound extraction could be justified by the temperature that has been reached during the two extraction methodologies. As reported by Zhang and coworkers [21] the solubility of naringenin gradually increases as the temperature increases; based on these results, it is expected to obtain lower extraction of these compounds during ultrasound extraction instead of that of infusion. This hypothesis can be confirmed with the results obtained by Wen et al. [22] that noticed that naringenin is insoluble in water at room temperature.

However, naringenin content in infusion samples reported a decreasing trend when increasing the time of infusion; these results should be attributed to a degradation of this compound when the thermal treatment was prolonged.

To the best of our knowledge, there is no literature about the water solubility and the effect of temperature on HHDP compound. Nevertheless, taking into account the results reported in Table 2, a similar trend to the one reported for naringenin compound could be supposed for HHDP.

Flavan-3-ols, gallic and ellagic acid derivatives, benzophenones, and flavonols in the ultrasound aqueous extract were from 3 to 5 times more concentrated than leaves infusions. Compared to the ultrasound aqueous extract (AE) and infusion of 7 min (I7) samples, naringenin was 1.5 and 1.7 times higher in the infusion of 3 min (I3) and in the 5 min (I5) samples, respectively.

Flavonols represent about 50 percent of total polar compounds in each sample. Avicularin and guajaverin were the major flavonol components and their concentrations varied from 13.7 to 3.2 mg/g and from 12.8 to 2.7 mg/g, respectively. Similar trend was showed by Chang et al. [11]. Morin was also found in high concentration with a range that varies from 3.0 to 8.4 mg/g in I7 and AE sample, respectively. Other flavonol compounds presented in all samples in higher quantities and in the same order of magnitude were hyperin, quercitrin,

reynoutrin, and isoquercitrin. Myricetin-arabinoside was detected in all samples, but it was quantified only in AE sample; instead, quercetin was only detected and quantified in the AE sample. These data could promote the use of guava leaves extract for nutraceutical scopes because, as reported by Wang et al. [23], myricetin and quercetin have high inhibitory activities against some enzymes that are involved in diabetes. Guavinoside C was quantified in ultrasound aqueous extract; it was identified in infusion samples, but its content was lower than LOQ.

The second class of polar compounds was represented by flavan-3-ols, which correspond to 26–30% of total polar compounds. Procyanidin was the first polar compound and its amounts ranged from 6.1 to 17.7 mg/g. Catechin was the second flavan-3-ol ranging between 5.1 and 12.9 mg/g.

Two epigallocatechin isomers and prodelpinidin dimer were the third flavan-3-ols and their amounts were about 5.4–5.9 mg/g.

Gallic and ellagic acid derivatives account for 20% of the total concentration of polar compounds in each sample. In this case, ultrasound aqueous extract and infusions reported different extraction power. Effectively, ultrasound aqueous extract showed casuarinin/casuarictin as first ellagic acid derivative (8.7 mg/g); on the contrary, infusion samples reported HHDP glucose compounds in the highest amounts (2.0–2.3 mg/g). Benzophenones were 2–4% of total polar compounds. Guavinoside A was the first benzophenone and it was represented by two isomers. Finally, four guavinoside B isomers were also detected in the extract, but only one was quantified; their content in infusion samples was less than LOQ or, in some cases, they were not detected.

At last, a flavanone, namely, naringenin was presented in all samples; I3 sample showed the higher content; on the contrary, aqueous extract and I7 samples reported the lowest quantities.

**3.3. Comparison between Phenolic Content and Antioxidant Activity.** As shown in Figure 1, the amount of total polar compounds is significantly higher in the ultrasound aqueous extract than in the infusions. Comparing the results obtained for the infusions, the quantity of these compounds is quite higher for I5 than for the others, I3 and I7. In fact, I3 sample reported a lower content probably due to an incomplete extraction of polar compounds; instead, I7 sample showed lower amounts, probably due to a degradation of these compounds during maceration.

To evaluate the antioxidant activity of the extract and to corroborate the correlation between phenolic content and antioxidant activity, two different assays were developed: TEAC evaluated by ABTS<sup>•+</sup> test and FRAP.

The choice of these two methods was assessed based on their different mechanisms: the radical scavenging capacity demonstrated by ABTS and ferric reducing capacities evaluable by FRAP method. Moreover, the results obtained by Thaipong et al. [24] demonstrated that ABTS and FRAP reported higher correlation with total phenolic content in guava fruit compared to other antioxidant activity assays.

Total polar compounds by HPLC are in concordance with the values obtained for the FRAP and ABTS assays

TABLE 2: Quantification (mean  $\pm$  SD,  $n = 3$ ) of the compounds identified in guava leaves infusions and ultrasound aqueous extract.

Number	Compounds	Quantification ( $\mu\text{g}$ analyte/g leaves)			
		AE	I3	I5	I7
1	HHDP glucose isomer	1146 $\pm$ 34 <sup>c</sup>	2256 $\pm$ 37 <sup>a</sup>	2253 $\pm$ 27 <sup>a</sup>	2021 $\pm$ 67 <sup>b</sup>
2	HHDP glucose isomer	228 $\pm$ 40 <sup>b</sup>	441 $\pm$ 72 <sup>a</sup>	368 $\pm$ 59 <sup>a,b</sup>	397 $\pm$ 93 <sup>a,b</sup>
3	HHDP glucose isomer	1424 $\pm$ 48 <sup>c</sup>	1756 $\pm$ 39 <sup>a</sup>	1762 $\pm$ 12 <sup>a</sup>	1546 $\pm$ 27 <sup>b</sup>
4	Gallic acid	719 $\pm$ 33 <sup>a</sup>	240 $\pm$ 5 <sup>b</sup>	260 $\pm$ 5 <sup>b</sup>	254 $\pm$ 9 <sup>b</sup>
5	Prodelphinidin B2 isomer	665 $\pm$ 55 <sup>a</sup>	212 $\pm$ 11 <sup>b</sup>	220 $\pm$ 12 <sup>b</sup>	126 $\pm$ 8 <sup>c</sup>
6	Pedunculagin/casuariin isomer	2405 $\pm$ 38 <sup>a</sup>	573 $\pm$ 14 <sup>c</sup>	688 $\pm$ 5 <sup>b</sup>	533 $\pm$ 6 <sup>c</sup>
7	Prodelphinidin dimer isomer	1768 $\pm$ 90 <sup>a</sup>	444 $\pm$ 26 <sup>b</sup>	467 $\pm$ 20 <sup>b</sup>	335 $\pm$ 11 <sup>b</sup>
8	Gallocatechin	5887 $\pm$ 273 <sup>a</sup>	2419 $\pm$ 19 <sup>b</sup>	2301 $\pm$ 43 <sup>b,c</sup>	1960 $\pm$ 77 <sup>c</sup>
9	Prodelphinidin dimer isomer	5452 $\pm$ 308 <sup>a</sup>	1526 $\pm$ 4 <sup>b</sup>	1688 $\pm$ 40 <sup>b</sup>	1309 $\pm$ 34 <sup>b</sup>
10	Geraniin isomer	1396 $\pm$ 47 <sup>a</sup>	304 $\pm$ 1 <sup>b</sup>	318 $\pm$ 12 <sup>b</sup>	201 $\pm$ 8 <sup>c</sup>
11	Pedunculagin/casuariin isomer	2894 $\pm$ 46 <sup>a</sup>	687 $\pm$ 9 <sup>c</sup>	814 $\pm$ 19 <sup>b</sup>	624 $\pm$ 6 <sup>c</sup>
12	Geraniin isomer	2333 $\pm$ 160 <sup>a</sup>	433 $\pm$ 26 <sup>b</sup>	457 $\pm$ 8 <sup>b</sup>	304 $\pm$ 3 <sup>b</sup>
13	Procyanidin B isomer	17659 $\pm$ 785 <sup>a</sup>	6703 $\pm$ 124 <sup>b</sup>	7106 $\pm$ 51 <sup>b</sup>	6105 $\pm$ 34 <sup>b</sup>
14	Procyanidin B isomer	1751 $\pm$ 150 <sup>a</sup>	398 $\pm$ 10 <sup>b</sup>	413 $\pm$ 10 <sup>b</sup>	321 $\pm$ 9 <sup>b</sup>
15	Tellimagrandin I isomer	728 $\pm$ 26 <sup>a</sup>	141 $\pm$ 2 <sup>c</sup>	184 $\pm$ 5 <sup>b</sup>	129 $\pm$ 2 <sup>c</sup>
16	Catechin	12875 $\pm$ 705 <sup>a</sup>	6127 $\pm$ 80 <sup>b</sup>	5960 $\pm$ 19 <sup>b</sup>	5192 $\pm$ 2 <sup>b</sup>
17	Casuarinin/casuarictin isomer	8725 $\pm$ 216 <sup>a</sup>	482 $\pm$ 1 <sup>c</sup>	859 $\pm$ 21 <sup>b</sup>	590 $\pm$ 17 <sup>b,c</sup>
18	Tellimagrandin I isomer	1492 $\pm$ 46 <sup>a</sup>	186 $\pm$ 6 <sup>b,c</sup>	243 $\pm$ 9 <sup>b</sup>	164 $\pm$ 3 <sup>c</sup>
19	Gallocatechin	5866 $\pm$ 362 <sup>a</sup>	2205 $\pm$ 134 <sup>b,c</sup>	2446 $\pm$ 21 <sup>b</sup>	1896 $\pm$ 13 <sup>c</sup>
20	Myricetin-arabinoside/xylopyranoside isomer	608 $\pm$ 18 <sup>a</sup>	<LOQ	<LOQ	<LOQ
21	Procyanidin B isomer 2	1206 $\pm$ 56 <sup>a</sup>	207 $\pm$ 14 <sup>b,c</sup>	224 $\pm$ 7 <sup>b</sup>	136 $\pm$ 6 <sup>c</sup>
22	Myricetin hexoside isomer	1301 $\pm$ 44 <sup>a</sup>	<LOQ	<LOQ	<LOQ
23	Myricetin hexoside isomer	331 $\pm$ 2 <sup>a</sup>	245 $\pm$ 2 <sup>c</sup>	299 $\pm$ 3 <sup>b</sup>	232 $\pm$ 4 <sup>d</sup>
24	Myricetin-arabinoside/xylopyranoside isomer	639 $\pm$ 29 <sup>a</sup>	<LOQ	<LOQ	<LOQ
25	Quercetin-galloylhexoside isomer	566 $\pm$ 20 <sup>a</sup>	148.3 $\pm$ 0.1 <sup>b</sup>	171 $\pm$ 2 <sup>b</sup>	149 $\pm$ 1 <sup>b</sup>
26	Quercetin-galloylhexoside isomer	452 $\pm$ 15 <sup>a</sup>	97.25 $\pm$ 0.02 <sup>b</sup>	102.3 $\pm$ 0.4 <sup>b</sup>	92.25 $\pm$ 0.07 <sup>b</sup>
27	Myricetin-arabinoside/xylopyranoside isomer	592 $\pm$ 24 <sup>a</sup>	<LOQ	<LOQ	<LOQ
28	Morin	8377 $\pm$ 464 <sup>a</sup>	3235 $\pm$ 53 <sup>b,c</sup>	3676 $\pm$ 12 <sup>b</sup>	3003 $\pm$ 18 <sup>c</sup>
29	Myricetin-arabinoside/xylopyranoside isomer	986 $\pm$ 28 <sup>a</sup>	<LOQ	<LOQ	<LOQ
30	Ellagic acid	4338 $\pm$ 234 <sup>a</sup>	1082 $\pm$ 16 <sup>b</sup>	1367 $\pm$ 14 <sup>b</sup>	1040 $\pm$ 9 <sup>b</sup>
31	Hyperin	7798 $\pm$ 280 <sup>a</sup>	2492 $\pm$ 6 <sup>c</sup>	2891 $\pm$ 7 <sup>b</sup>	2031 $\pm$ 18 <sup>d</sup>
32	Quercetin glucuronide	2293 $\pm$ 91 <sup>a</sup>	1249 $\pm$ 19 <sup>c</sup>	1612 $\pm$ 40 <sup>b</sup>	1132 $\pm$ 7 <sup>c</sup>
33	Isoquercitrin	4408 $\pm$ 182 <sup>a</sup>	1111 $\pm$ 19 <sup>b,c</sup>	1306 $\pm$ 5 <sup>b</sup>	996 $\pm$ 3 <sup>c</sup>
34	Reynoutrin	5849 $\pm$ 173 <sup>a</sup>	1386 $\pm$ 21 <sup>b,c</sup>	1611 $\pm$ 20 <sup>b</sup>	1229 $\pm$ 9 <sup>c</sup>
35	Guajaverin	12843 $\pm$ 421 <sup>a</sup>	3169 $\pm$ 40 <sup>b,c</sup>	3595 $\pm$ 4 <sup>b</sup>	2713 $\pm$ 36 <sup>c</sup>
36	Guavinoside A	1920 $\pm$ 40 <sup>a</sup>	453 $\pm$ 3 <sup>c</sup>	522 $\pm$ 1 <sup>b</sup>	413 $\pm$ 2 <sup>c</sup>
37	Avicularin	13666 $\pm$ 421 <sup>a</sup>	3825 $\pm$ 48 <sup>b</sup>	4183 $\pm$ 34 <sup>b</sup>	3232 $\pm$ 25 <sup>c</sup>
38	Quercitrin	6822 $\pm$ 274 <sup>a</sup>	1825 $\pm$ 39 <sup>b</sup>	2078 $\pm$ 24 <sup>b</sup>	1705 $\pm$ 12 <sup>b</sup>
39	Guavinoside C	2298 $\pm$ 40 <sup>a</sup>	<LOQ	<LOQ	<LOQ
40	Guavinoside B	1456 $\pm$ 34 <sup>a</sup>	370 $\pm$ 3 <sup>c</sup>	423 $\pm$ 4 <sup>b</sup>	328.6 $\pm$ 0.1 <sup>c</sup>
41	Guavinoside A isomer	558 $\pm$ 4 <sup>a</sup>	177 $\pm$ 1 <sup>b</sup>	160 $\pm$ 4 <sup>c</sup>	140.0 $\pm$ 0.3 <sup>d</sup>
42	Prodelphinidin B2 isomer 2	<LOQ	<LOQ	<LOQ	<LOQ
43	Guavinoside B isomer	<LOQ	<LOQ	<LOQ	<LOQ
44	Guavinoside B isomer	<LOQ	nd	nd	nd
45	Guavinoside B isomer	<LOQ	nd	nd	nd
46	Guavin B	460 $\pm$ 6 <sup>a</sup>	34 $\pm$ 1 <sup>b</sup>	32.2 $\pm$ 0.2 <sup>b</sup>	20 $\pm$ 1 <sup>c</sup>
47	Quercetin	408 $\pm$ 18 <sup>a</sup>	nd	nd	nd
48	Naringenin	538 $\pm$ 14 <sup>c</sup>	941 $\pm$ 3 <sup>a</sup>	815 $\pm$ 24 <sup>b</sup>	558 $\pm$ 9 <sup>c</sup>

n.d.: not detected; AE: aqueous extract obtained by ultrasound; I3, I5, and I7: infusion obtained at 3, 5, and 7 minutes of infusion time, respectively. The different letter in the same line means that the compounds are significantly different ( $P \leq 0.05$ ).

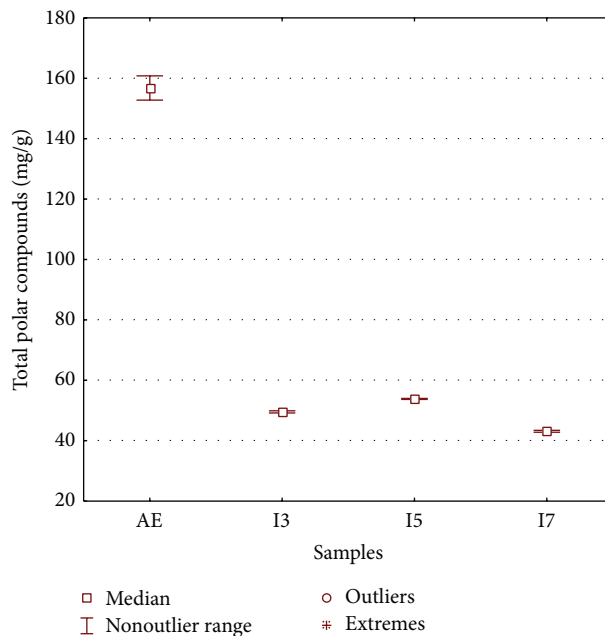


FIGURE 1: Total content (mg/g) of total polar compounds by HPLC in analysed samples.

TABLE 3: Comparison between total polar compound (mg/g) determined by HPLC and antioxidant activity evaluated by FRAP ( $\mu\text{M}$  of  $\text{FeSO}_4$  equivalents/mg) and ABTS ( $\mu\text{M}$  of Trolox equivalents/mg).

Sample	TPC by HPLC	FRAP	ABTS
AE	157 ± 6.0 <sup>a</sup>	3026.7 ± 586.4 <sup>a</sup>	1127.6 ± 69.0 <sup>a</sup>
I3	49.6 ± 0.5 <sup>c</sup>	314.2 ± 15.1 <sup>b</sup>	155.7 ± 1.1 <sup>b</sup>
I5	53.8 ± 0.2 <sup>b</sup>	285.3 ± 7.0 <sup>b</sup>	217.6 ± 19.2 <sup>b</sup>
I7	43.1 ± 0.4 <sup>d</sup>	285.7 ± 7.0 <sup>b</sup>	178.2 ± 1.3 <sup>b</sup>

Means in the same column with different letter are significantly different ( $P < 0.05$ ).

(Table 3). Besides, the reducing power and radical scavenging capacity displayed a significant difference between the samples obtained by infusion and the ultrasound aqueous extract. Positive correlations with  $R = 0.9883$  and  $P < 0.001$  and  $R = 0.9973$  and  $P < 0.001$  were noticed between total polar compounds content and FRAP and ABTS, respectively.

FRAP and ABTS did not report significant differences ( $P < 0.05$ ) among infusion samples; however, ultrasound aqueous extract values were higher than infusions values.

Moreover, a simple linear regression analysis was carried out to compare the correlation between all compounds identified and the antioxidant activity (Table 4).

Most of the polar compounds were highly correlated with FRAP assay ( $R = 0.98$ ;  $P < 0.001$ ) except compounds 10, 30, and 32 that reported an  $R$  value ranging between 0.96 and 0.97 ( $P < 0.001$ ). Compound 23 showed a lower correlation ( $R = 0.76$ ;  $P < 0.05$ ). HHDP glucose isomers resulted in inverse correlation with FRAP assay. Moreover, naringenin did not show any correlation.

ABTS assay confirmed data reported by FRAP assay; in fact, the two antioxidant assays showed a good correlation

between them that reported an  $R$  value of 0.9916 and  $P < 0.0001$ . These results agreed with the data reported by Thaipong et al. [24].

#### 4. Conclusions

Several polar compounds have been identified and quantified in guava leaves extracts (ultrasound aqueous extract and infusions). According to the amount of polar compounds and also the FRAP and ABTS assays, the water ultrasound assisted extraction provided better results than the infusion. Significant positive correlations  $R > 0.98$  and  $P < 0.001$  were detected between total polar content and antioxidant activity assays. Moreover, positive correlation was also detected for single compounds, except for HHDP and naringenin.

The results suggested that aqueous ultrasound extract can represent a valuable strategy to obtain nutraceuticals using a green technology. About infusions, the 5-minute infusion is advisable for guava leaves culinary uses because of reported higher polar compounds content.

TABLE 4: Correlation between the antioxidant activity and polar compounds.

	Compounds	FRAP		ABTS	
		R value	P value	R value	P value
1	HHDP glucose isomer	-0.9644	***	-0.9721	***
2	HHDP glucose isomer	-0.8199	*	-0.8321	*
3	HHDP glucose isomer	-0.7507	*	-0.7700	*
4	Gallic acid	0.992	***	0.9993	***
5	Prodelphinidin B2 isomer	0.9817	***	0.9845	***
6	Pedunculagin/casuariin isomer	0.9849	***	0.9980	***
7	Prodelphinidin dimer isomer	0.9895	***	0.9962	***
8	Gallocatechin	0.9887	***	0.9922	***
9	Prodelphinidin dimer isomer	0.9909	***	0.9977	***
10	Geraniin isomer	0.9747	***	0.9911	***
11	Pedunculagin/casuariin isomer	0.985	***	0.9979	***
12	Geraniin isomer	0.9922	***	0.9911	***
13	Procyanidin B isomer	0.9907	***	0.9978	***
14	Procyanidin B isomer	0.9949	***	0.9973	***
15	Tellimagrandin I isomer	0.987	***	0.9985	***
16	Catechin	0.99	***	0.9916	***
17	Casuarinin/casuarictin isomer	0.9874	***	0.9990	***
18	Tellimagrandin I isomer	0.9881	***	0.9989	***
19	Gallocatechin	0.9881	***	0.994	***
20	Myricetin-arabinoside/xylopyranoside isomer	0.9889	***	0.9978	***
21	Procyanidin B isomer 2	0.9891	***	0.9967	***
22	Myricetin hexoside isomer	0.9893	***	0.9979	***
23	Myricetin hexoside isomer	0.7694	*	0.8073	*
24	Myricetin-arabinoside/xylopyranoside isomer	0.9907	***	0.9981	***
25	Quercetin-galloylhexoside isomer	0.9893	***	0.9994	***
26	Quercetin-galloylhexoside isomer	0.99	***	0.9986	***
27	Myricetin-arabinoside/xylopyranoside isomer	0.9901	***	0.9980	***
28	Morin	0.9889	***	0.9967	***
29	Myricetin-arabinoside/xylopyranoside isomer	0.9887	***	0.9977	***
30	Ellagic acid	0.9684	***	0.9915	***
31	Hyperin	0.983	***	0.9938	***
32	Quercetin glucuronide	0.9622	***	0.9794	***
33	Isoquercitrin	0.9887	***	0.9983	***
34	Reynoutrin	0.9874	***	0.9982	***
35	Guajaverin	0.9878	***	0.9978	***
36	Guavinoside A	0.9866	***	0.9982	***
37	Avicularin	0.9874	***	0.9970	***
38	Quercitrin	0.9897	***	0.9989	***
39	Guavinoside C	0.9873	***	0.9974	***
40	Guavinoside B	0.9864	***	0.9979	***
41	Guavinoside A isomer	0.9842	***	0.9928	***
46	Guavin B	0.9865	***	0.9968	***
47	Quercetin	0.9906	***	0.9981	***
48	Naringenin	-0.5619	NC	-0.5855	NC
	TPC by HPLC	0.9883	***	0.9973	***

\*\*\*  $P < 0.001$ ; \*  $P < 0.05$ ; NC: not correlated.



## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Acknowledgments

This work was funded by the Project cofinanced by FEDER-Andalucía 2007–2013 (Cod. 461100) and Andalusian Regional Government Council of Innovation and Science (P11-CTS-7625). The author Elixabet Díaz-de-Cerio also would like to thank the CEIBiotic for the “Ayudas a la Enseñanza Práctica” Grant (CADP2-71). Ana María Gómez-Caravaca and Vito Verardo thank the Spanish Ministry of Economy and Competitiveness (MINECO) for “Juan de la Cierva” postdoctoral contract.

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