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Assessment of antioxidant and antibacterial potential of borojo fruit (*Borojoa patinoi* Cuatrecasas) from the rainforests of South America

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ABSTRACT

Borojo (*Borojoa patinoi*) is an endemic fruit of the rainforests of Colombia, Brazil, Peru, Ecuador and Panama that is listed for approval as novel food in the European Union. In this work the antioxidant activity (measured with DPPH, FIC and FRAP), the organic acid and sugar contents, the volatile profile and the antibacterial properties against 26 pathogenic and non-pathogenic strains were studied. Total phenol contents (TPC) ranged from 36.41 to 53.6 mg GAE/100 g fw while total flavonoids (TFC) from 88.45 to 49.83 mg RE/100 g fw. The extract showed also good antioxidant activity highly correlated to TPC and TFC. Among 21 volatile compounds detected by SPME–GC–MS, 2-nonanol represented the main component. The antimicrobial activity was in a decreasing order: *Salmonella enteritidis* > *Salmonella typhimurium* > *Listeria monocytogenes* > *Staphylococcus aureus* > *Brochotrix thermosphacta*. The biological properties of Borojo-fruit suggested that it is a promising new antioxidant and antibacterial agent.

1. Introduction

In South America, mainly Colombia, Brazil, Ecuador or Peru, a wide range of fruits from tropical and subtropical areas exist with different degrees of importance to the fruit sector, depending on their economic and social impact. Many of these fruits are produced and consumed in local markets and are rarely exported, often because of their perishability and the lack of knowledge of their sensory properties and nutritional value.

Borojo (*Borojoa patinoi* Cuatrecasas) is the name of a plant species belonging to the *Rubiaceae* family and endemic to the rainforests of Colombia, Brazil, Ecuador and Panama. The fruit is an edible berry 7–12 cm in length, generally flat at the apex, green in colour at the beginning and light brown at maturity; pulp constituted mesocarp and the endocarp, with no apparent separation from the shell, weight between 740 and 1000 g, with an average of 330 seeds per fruit. The pulp represents 88% of the total fruit weight, the water is about 64% and the water activity near to 0.91 (Sotelo et al., 2010). It exhibits an intense floral aroma and sweet acid taste and may be stored for refrigeration or at room temperature for up to 6 months (Diaz-Ocampo et al., 2012). Borojo pulp is used to prepare processed products like jelly, sauces, marmalades and juices. This fruit is also used in the traditional medicines with supposed antihypertensive, antitumoral, diuretic, healing and aphrodisiac effects (Gentry, 1988). It is also used by the local communities against bronchial diseases, gastritis and malnutrition.

Colombia produces nearly 17,000 t of borojo and the consumption of this fruit is increasing in both domestic and international markets due to the growing recognition of its value to human health (Contreras-Calderón et al., 2011); thus, the dynamic exportation of this fruit is linked to the new preferences of the international markets. In fact, this fruit has been famous for its health properties. For this reason, it is a special source of income for some of the native population who sell it in the local food markets in the main Colombian cities. Actually, it is listed for approval as novel food in the European Union. Studies have shown that borojo pulp has a pH of 2.9 and contains fat (0.15%), protein (0.69–0.78%), dietary fibre (23.58%), vitamins (C, B2, B3) and minerals (P, Fe, Ca, K) (Mosquera et al., 2010; Diaz-Ocampo et al., 2012,). Moreover, the presence of organic acids such as ascorbic, oxalic acid in the pulp has been also reported (Contreras-Calderón et al., 2011). On the other hand, triterpenes, flavonoids and phenols have been reported, in particular the total phenol content is ranged between 28 mg of GAEs/100 g and 253 mg of GAEs/100 g (Sotelo et al., 2010; Contreras-Calderón

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et al., 2011). In a study conducted by Toledo-Romaneiko (2009) on six different borojo clones, it has been evidenced that the nutritional composition of borojo depends on the type of clone studied; in fact, values of Fe ranged between 22 and 70 mg/kg, vitamin A between 764 and 3015 UI/100 g and vitamin C between 14 and 22 mg/100 g; also total phenols content ranged between 39.6 and 112 mg of GAEs/100 g.

Besides the healthy promoter characteristics, borojo has showed antimicrobial activity towards *Escherichia coli* and *Staphylococcus aureus*, and this activity has been attributed to the presence of polyphenols (Sotelo et al., 2010). Actually in Colombia there is an interest to generate high value-added products from borojo which maintain nutritional properties of the pulp or seed. Thus, the recovery of valuable compounds from natural resources is nowadays conducted using the so called "5-stage universal recovery processing" (Galanakis, 2012).

The aims of the present work were to determine (i) the organic acid and sugar contents, (ii) the volatile profile and (iii) to evaluate the antioxidant and antibacterial properties of borojo (*B. patinoi* Cuatrecasas) fruit from the rainforests of Colombia.

2. Materials and methods

2.1. Plant material

A total of twelve Borojo (*B. patinoi*) fruits with no visible external cuts or spoilage were purchased from a local market in Cali (Colombia).

2.2. Sample preparation

Borojo fruits were cleaned with tap water and then separated into peel and pulp (the edible part) and seeds (the non-edible part). Immediately, the edible portion was chopped and homogenized for 10 s whilst the non-edible portion was discarded. The time between chopping the fruit and beginning the extraction was 5 min.

To obtain the extracts, three different methodologies were used. In the first procedure 10 g of the edible part of borojo were placed in a capped centrifuge tube and 30 mL of methanol–water (80–20, v/v) were added, after which the mixture was homogenized in an Ultra-Turrax (IKA, T25D, Staufen, Germany) during 3 min at 18,000 rpm. The tube was then centrifuged at 2739 × *g* for 20 min at 4 °C and the supernatant was transferred to a round-bottomed flask and evaporated to dryness using a rotary evaporator R-205 (Büchi, Flawil, Switzerland) under reduced pressure (<100 mbar) at 40 °C. Five millilitres of methanol were added to the residue, and the mixture was well shaken in a Vortex for 2 min.

In the second procedure, ten grams of borojo samples were mixed with 30 mL of ethanol, vortexed for 1 min and homogenized in an Ultra-Turrax during 3 min at 18,000 rpm. The tube was then centrifuged at 2739 × *g* for 20 min at 4 °C and the supernatant was recovered. Thirty millilitres of acetone–water (70:30, v/v) were added to the residue, followed by shaking, homogenizing and centrifugation. Supernatants were combined and evaporated to dryness using a rotary evaporator R-205 under reduced pressure at 40 °C. Five millilitres of methanol were added to the residue, and the mixture was well shaken in a Vortex for 2 min.

In the third procedure, ten grams of borojo fruit were extracted with 10 mL of water using an ultrasonic water bath (Selecta S.A. Barcelona, Spain) without temperature control, during 3 h. Then, the mixtures were centrifuged at 2739 × *g* for 20 min at 4 °C. After centrifugation supernatants were filtered through a 0.45 μm Millipore filter (Millipore Corporation, Bedford, USA).

The extracts obtained were stored at −20 °C and measured before 24 h. The three fractions obtained were: borojo extracted with methanol (BE_M), borojo extracted with ethanol:acetone (BE_{EA}) and borojo extracted with water (BE_w).

2.3. Total phenol content

To determine the total phenol content (TPC) of borojo extracts the Folin-Ciocalteu's reagent (Singleton and Rossi, 1965) was used. The results were expressed as mg Gallic acid equivalents (GAE)/100 g sample (fresh weight (fw)). Each assay was carried out in triplicate.

2.4. Total flavonoid content

For the total flavonoid content (TFC), the method described by Blasa et al. (2005) was used. The results were expressed in mg rutin equivalents (RE)/100 g of sample (fw) as mean of three replicates.

2.5. Determination of polyphenolic compounds

Twenty microliters of the different extracts (BE_M, BE_{EA} and BE_w) were injected into a Hewlett-Packard HPLC series 1100 instrument (Woldbronn, Germany) equipped with UV-Vis Diode Array Detector. Separations were realized on a C_{18} Teknokroma column (Mediterranea sea_{18}, 25 cm × 0.4 cm, 5 μm particle size, Teknokroma, Barcelona, Spain). Spectral data from all peaks were accumulated in the range 200–400 nm, and the chromatograms were recorded at 280, 320 or 360 nm. Phenolic compounds were analyzed, in standard and sample solutions, using a gradient elution at 1 mL/min with the following gradient programme, started with 95% A, 75% A at 20 min, 50% A at 40 min, 20% A at 50 min and 20% A at 60 min. The mobile phase was composed by formic acid:water (4.5:95.5) (A) and acetonitrile as solvent B according to a described procedure (López-Vargas et al., 2013). The quantitative analysis of the components was achieved with reference to authentic standards (phenolic acid standards: catechin, epicatechin, caffeic, ferulic, synapic, *p*-coumaric, gallic, chlorogenic acids; flavonoids standards: rutin, quercetin, luteolin, apigenin and luteolin-7-*O*-glucoside) (Extrasynthese, Genay, France). Compound identification was carried out by comparing UV absorption spectra and retention times of each compound with those of pure standards injected in the same conditions. The compounds were quantified through calibration curves of standard compounds as mean of three replicates.

2.6. Antioxidant activity

2.6.1. Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) of the different borojo extracts was determined by using the potassium ferricyanide–ferric chloride method, as described by Oyaizu (1986). The ferric reducing activity of a sample was estimated in μM Trolox equivalent (TE)/100 g of sample (fw). Each assay was carried out in triplicate.

2.6.2. Ferrous ion-chelating ability assay

Ferrous ions (Fe^{2+}) chelating activity was measured by inhibiting the formation of Fe^{2+}–ferrozine complex after treatment of test material with Fe^{2+}, following the method of Carter (1971). Results were expressed in μM EDTA equivalent/100 g sample (fw) as mean of three replicates.

2.6.3. DPPH radical scavenging ability assay

The radical scavenging ability of the different extracts obtained from borojo was measured using the stable radical DPPH

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(Brand-Williams et al., 1995). Results were expressed in μM Trolox equivalent (TE)/100 g of sample (fw) as mean of three replicates.

2.7. Organic acid and sugar content

2.7.1. Extraction of organic acid and sugars

One gram of borojo samples was homogenized with 10 mL of acidified ultrapure water (0.1% phosphoric acid) in an Ultra-Turrax at 13,500 rpm for 20 s. Then, the samples were centrifuged at 5000 × *g* for 10 min at 4 °C and the supernatants were filtered through 0.45 μm Millipore filter (Millipore Corporation). Triplicate extractions were obtained from each sample.

2.7.2. HPLC analysis

Organic acids and sugars were analyzed in a Hewlett Packard HP-1100 instrument (Woldbronn, Germany) coupled with two detectors: UV-Vis Diode Array Detector G1315A (set at 210 nm) and refractive index detector G-1362. Twenty microliters of sample were injected in a cation exchange column (Supelcogel C-610 H, 300 mm × 7.8 mm, Supelco, Bellefonte) with a pre-column (Supelguard-H, 50 mm × 4.6 mm, Supelco), using phosphoric acid (0.1%) as mobile phase, operating flow rate of 0.5 mL/min. Samples were run at 30 °C and the run time was 30 min (Doughty, 1995). Standards of organic acids (L-ascorbic, malic, tartaric, citric, oxalic, acetic, malonic, lactic, fumaric and succinic acids) and monosaccharides (maltose, glucose, fructose and sucrose) were obtained from Sigma (Poole, Dorset, UK). Peaks were identified by comparison with retention time of the standards, and quantified by regression formula obtained with the standards.

2.8. Volatile compounds

2.8.1. Extraction procedure

Headspace solid phase micro-extraction (HS-SPME) was the method selected to study the volatile composition of borojo fruits. After several preliminary tests to optimize the extraction system, 10 mL of pulp were hermetically placed into 50 mL vials with polypropylene caps and PTFE/silicone septa; the ratio pulp to headspace was approximately 1:4. A magnetic stirring bar was added, together with NaCl (15%: 1.5 g per 10 mL) and the vial was placed in a water bath with temperature control and stirring. Vials were equilibrated during 15 min at 40 °C in the bath and after this equilibration time, a 50/30 μm DVB/CAR/PDMS fibre was exposed to the sample headspace for 50 min at 40 °C. This type of fibre was chosen for its high capacity of trapping fruits volatile compounds. After sampling, the desorption of the volatile compounds from the fibre coating was carried out in the injection port of the GC–MS during 3 min.

2.8.2. Chromatographic analysis

The isolation and identification of the volatile compounds were performed on a gas chromatograph, Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector GC-MS QP-5050A. The GC–MS system was equipped with a TRACSIL Meta.X5 column, 95% dimethyl-polysiloxane and 5% diphenyl-polysiloxane (Teknokroma S. Coop. C. Ltd, Barcelona, Spain; 60 m × 0.25 mm × 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a column flow of 0.6 mL/min in a split ratio of 1:5 and the following programme: (i) 80 °C for 0 min; (ii) rate of 3.0 °C/min from 80 to 210 °C and hold for 1 min; (iii) rate of 25 °C/min from 210 to 300 °C and hold for 3 min. The temperatures of the injector and detector were 230 and 300 °C, respectively. Most of the compounds were identified by using 3 different analytical methods: (i) retention indices (KI), (ii) GC–MS retention times (authentic chemicals), and (iii) mass spectra (authentic chemicals and NIST05 spectral library collection) (NIST, 2010). Identification was considered tentative when it was based on only mass spectral data. The volatile studies were conducted in triplicate.

2.9. Strains

The strains used in this study were *Listeria monocytogenes* (5 strains: LM15, LM16, LM49; LM56 and ATCC 19144), *S. aureus* (5 strains: STA59393, STA39533; CF025, CF032 and CP2801), *Salmonella typhimurium* (8 strains: S1, S3, S4, S5, S6, ST37, ST44 and ST56), *Salmonella enteritidis* (2 strains: SE2 and SE4406) and *Brochotrix thermosphacta* (4 strains: B01, B02, B03 and B04). Except ATTC strains, all belonged to the Faculty of Bioscience and Technology for Food, Agriculture and Environment of Teramo University. The cultures were grown at 37 °C to stationary phase (24 h) in Brain-Heart Infusion Broth (BHI, OXOID, Basingstoke, UK). This broth was then used to inoculate the final broth under adjustment of 10^3 CFU/mL. The culture was incubated at 37 °C up to stationary phase (20 h). The cells were harvested by centrifugation at 2739 × *g* for 10 min at 25 °C, washed twice with phosphate buffer saline 50 mM; pH 7.0 (PBS) and subsequently resuspended in PBS to obtain a final concentration of 8.5 log CFU/mL.

2.10. Disc diffusion assay for inhibitory activity

Cultures (0.25 mL) of each test pathogen were spread on the surface of the plates containing BHI agar. Sterile paper discs (5.4 mm diameter) were immersed in diluted sterilized borojo solutions (1:3 borojo/water), blotted, and placed on the surface of inoculated BHI. Five discs were applied to each plate. After incubation at 37 °C for 24 h, zones of inhibition surrounding discs were measured with a dial calliper (0.1 mm accuracy). The diameter of zones, including the diameter of the disc, was recorded. The borojo solution was previously centrifuged (2739 × *g* for 10 min at 4 °C) and sterilized by filtration (0.22 μm). Five repetitions of all the experiment were performed.

2.11. Minimal inhibitory concentration MIC determination

Broth microdilution method was used to determine the MIC using a sterile 96 well microliter tray with lid. Water borojo extract (300 μL) at concentrations of: 30 (w/v), 20, 15, 10, 5, 2.5 and 0% (w/v) were tested and 2,3,5-triphenyltetrazolium chloride (Sigma, St. Louis, USA) supplemented with 0.01% (w/v) was used for the visual indicator of bacterial growth.

An aliquot (30 μL) containing 6 log CFU/mL of bacterial cells were inoculated in each well and incubated aerobically without agitation at 37 °C for 48 h. After this period bacterial growth was evidenced by the presence of red colour. Viability was confirmed by plating count. The MIC was defined as the lowest concentration of extract that completely inhibited visible bacterial growth. Each experiment was performed in five repetitions.

2.12. Time kill kinetics

Based on the results of disc diffusion assays, four strains (*S. enteritidis* SE4406, *L. monocytogenes* ATCC 19114, *B. thermosphacta* BT4 and *S. aureus* STA 39533), which showed the major sensitivity to borojo extract, were further studied for the time kill kinetics (TKK) in BHI broth. Aliquot (25 mL) of borojo extract was inoculated with a single culture of the strain with 8.5 ± 0.5 and 8.0 ± 0.5 log CFU/mL for Gram-positive and Gram-negative bacteria, respectively. The cell suspension was incubated at 37 °C for 24 h, and periodically (0, 3, 7, 12, 18, 24 h) an aliquot of 1 mL was sampled to assess the

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microbial counts in plates containing BHI agar. Plates were inoculated with 0.1 mL of a series of 10-fold dilutions in PBS of control and treated samples. After incubation at 37 °C for 48 h, the colonies were counted. All microbiological tests were repeated in three different experiments. Each experiment was performed in triplicate.

2.13. Statistical assay

Statistical analysis and comparisons among means were carried out using the statistical package SPSS 19.0 (SPSS Inc., Chicago, IL). The data collected for the antioxidant activity were analyzed by one-way analysis of variance to test the effects of extracting agent (levels: methanol, ethanol:acetone and water). The data collected for the antimicrobial activity were analyzed by two-way analysis of variance to test the effects of two fixed factors: bacteria strains and pH. Tukey's *post hoc* test was applied for comparisons of means; differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Total phenolic and total flavonoid content

Polyphenolic compounds are very important fruit constituents which can be used as an important indicator of antioxidant capacity. Additionally, they can be used as a preliminary screen for any product when intended as a natural source of antioxidants (Viuda-Martos et al., 2010). Fig. 1A shows the total phenolic content (TPC) of the edible part of borojo extracted with different solvents. The samples extracted with ethanol:acetone had higher ($P < 0.05$) TPC than the samples extracted with methanol with values of 53.23 and 36.41 mg GAE/100 g fw, respectively. These values were similar than that reported by Contreras-Calderón et al. (2011) (41.8 mg GAE/100 g fw) in borojo pulp cultivated in Colombia. On the other hand, Jiménez et al. (2014) informed that the aqueous extract of green borojo had a total phenol content of 284.02 mg GAE/100 g fw. It should be note that the variability of polyphenols content in fruits may be due to different growing conditions, cultivars, climate, geography, and maturation stage. This variation can have functional implications, as long as the polyphenols bioavailability is not low.

The total flavonoid contents (TFC) of the edible part of borojo fruits, expressed as mg rutin equivalent/100 g fw are presented in Fig. 1B. As in TPC, the samples extracted with ethanol:acetone had higher ($P < 0.05$) TFC than the samples extracted with methanol or water with values of 88.45, 49.86 and 16.58 mg RE/100 g fw, respectively.

To the best of our knowledge, there are no studies, as regards the TFC present in borojo fruits. In the last years, epidemiological studies have suggested that regular consumption of flavonoid-rich foods and beverages is associated with a decreased risk of cardiovascular diseases (Toh et al., 2013).

Correlation analysis was performed on the polyphenolic content analysis methods for the edible part of borojo fruits. High correlation between TPC and TFC assays was obtained ($r = 0.999$). From the results shown in Fig. 1A and B, it is evident that the recovery of phenolic compounds was dependent on the solvent used and its polarity. These results are in agreement with Alothman et al. (2009) who reported that the recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process.

3.2. Determination of polyphenolic profile

The HPLC analysis of borojo extracted with ethanol, methanol:acetone or water, by comparison with UV-spectra of authentic standards showed four main peaks, identified as catechin, epicatechin, ferulic acid and chlorogenic acid.

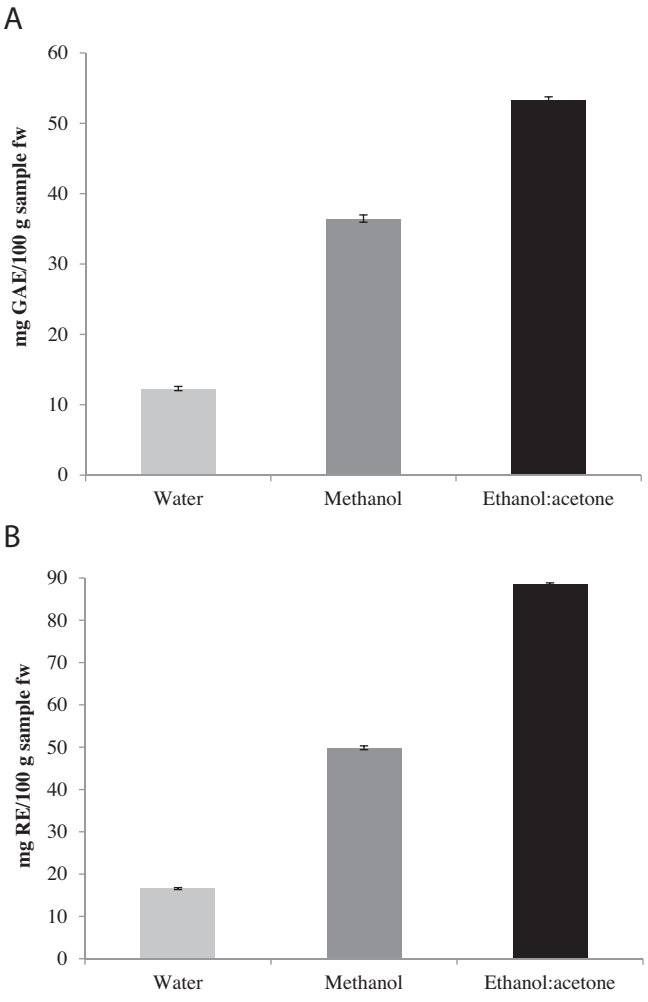

Fig. 1. (A) Total phenol content (TPC) expressed as mg Gallic acid equivalent (GAE) and (B) total flavonoid content (TFC) expressed as mg Rutin equivalent (RE) of borojo (*Borojoa patinoi*) fruit extracted with different solvents.

The samples extracted with ethanol:acetone showed higher content ($P < 0.05$) in polyphenolic compounds than samples extracted with methanol or water. BE_{EA} had the highest ($P < 0.05$) values for catechin (29.89 μg/g), epicatechin (18.93 μg/g) ferulic acid (34.22 μg/g) and chlorogenic acid (34.10 μg/g) followed by BE_M with values, for the same compounds of 16.18, 10.57, 24.14 and 22.09 μg/g respectively. In BE_w catechin and epicatechin were not found, while ferulic acid and chlorogenic acid had values of 8.21 and 11.23 μg/g respectively. This is the first study where the polyphenolic profile of borojo extracts was determined.

3.3. Antioxidant activity

Since the antioxidant activity of foodstuff is determined by a combination of different antioxidants with different action mechanisms, among which synergistic interactions, it is necessary to combine more than one method in order to determine *in vitro*, the antioxidant capacity of foods (Pérez-Jiménez et al., 2008). Additionally, there are considerable differences in sample preparation and method used for antioxidants extraction, the selection of endpoints and the mode in which the results are expressed, even for the same method (Viuda-Martos et al., 2010). In this work, three different methods were used to establish the antioxidant activities of borojo extracts. Table 1 shows the antioxidant activity of borojo fruit extracts using each of the FRAP, DPPH and FIC assays.

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Table 1
Antioxidant activity of borojo (*Borojoa patinoi*) fruits extracted with two different solvents and measured with three different methods.

Sample	Extraction	Antioxidant assay		
		DPPH (μM TE/100 g)	FRAP (μM TE/100 g)	FIC (μM EDTAE/100 g)
Borojo	Methanol	45.87 ± 0.81[b]	259.60 ± 10.14[b]	1.47 ± 0.02[b]
	Ethanol:acetone	55.55 ± 0.66[a]	426.58 ± 7.26[a]	1.63 ± 0.01[a]
	Water	11.32 ± 0.13[c]	102.74 ± 5.32[c]	0.21 ± 0.04[c]

TE: Trolox equivalent; EDTAE: ethylenediaminetetraacetic acid equivalent.
Values followed by the same small letter within the same column are not significantly different ($P > 0.05$) according to Tukey's Multiple Range Test.

The FRAP assay is frequently used to analyze the antioxidant ability of plant materials. The antioxidant capacity of fruits extracts is determined by the ability of the antioxidants in these extracts to reduce ferric to ferrous iron. The borojo samples extracted with ethanol:acetone showed higher ($P < 0.05$) ferric reducing capacity than the samples extracted with methanol or water.

The DPPH assay involved the measurement of colour loss. This assay is normally based on the scavenging of radical DPPH, converting it to a colourless product. The degree of this discoloration affects the quantity of DPPH that has been scavenged. Again, the borojo samples extracted with ethanol:acetone showed higher ($P < 0.05$) scavenging capacity than the samples extracted with methanol or water.

Ferrous ion, usually found in food products, is well known as an effective pro-oxidant agent. Polyphenolic compounds showed the property to chelate pro-oxidant metal ions, such as iron and copper and consequently avoiding free radical formation from these pro-oxidants. As in FRAP and DPPH assays borojo extracts extracted with ethanol:acetone had higher chelating effect ($P < 0.05$) than the borojo samples extracted with methanol or water.

To the best of our knowledge, there are few scientific works where the antioxidant activity of borojo extracts was determined. However, the antioxidant activity of borojo extracts was lower than what has been reported for other underutilized exotic fruits (Vasco et al., 2008; Contreras-Calderón et al., 2011). Several studies have revealed that most antioxidant activities arise from phenolic acids, flavonoids, anthocyanins, catechins and other phenolics compounds (Isabelle et al., 2010). In this work significant correlation between TPC or TFC and antioxidant capacity of fruits extracts (FRAP, FIC and DPPH values) was obtained. Thus, the correlations TPC-DPPH, TPC-FRAP and TPC-FIC were $r = 0.983$; $r = 0.998$ and $r = 0.996$ respectively, whilst the correlations TFC-DPPH, TFC-FRAP and TFC-FIC were $r = 0.986$; $r = 0.997$ and $r = 0.997$ respectively. In the same way, in the present work, the correlations for the DPPH-FRAP, DPPH-FIC and FRAP-FIC methods were $r = 0.973$; $r = 0.983$ and $r = 0.993$, respectively, indicating that borojo extract has comparable activity in all determinations. Several studies reported the relationships between phenolic content and antioxidant activity; some authors found a high correlation between the phenolic content and the antioxidant activity (Mezadri et al., 2008). However, it should be borne in mind that the antioxidant activity of fruit extracts is not the result of phenolic compounds alone. Other constituents, such as ascorbates, reducing carbohydrates, tocopherols, carotenoids, terpenes, and pigments, might contribute to the total antioxidant activity (Babbar et al., 2011).

3.4. Organic acid and sugar content

Table 2 shows the organic acid and sugar contents of borojo fruit. Organic acids are primary metabolites, which can be found in great amounts in all plants, especially in fruits. The organic acid profile of borojo was composed by five organic acids: oxalic, citric, tartaric, malic and L-ascorbic. The predominant organic acid was tartaric acid with values of 1.29 g/100 g sample fw. Malic and citric acids were also present in significant amount (0.73 and 0.48 g/100 g sample fw respectively). These two carboxylic acids behave as antioxidants since they have the ability to chelate metals (Seabra et al., 2006). As far as we know, this is the first time that organic acid profile is described in this fruit. Nevertheless, the organic acid contents in tropical fruits are extensively studied. Therefore, Sidhu (2006) reported that the organic acids (0.3–0.8%) contributing to acidity in Guava are mainly citric, malic, glycolic, tartaric and lactic acids.

With reference to sugar contents, the four sugars found in borojo fruits (Table 2) were fructose, maltose, glucose and sucrose in that order. The ratio glucose/fructose was 0.45. The content of fructose was relatively high, which is valuable of attention because this sugar is twice sweeter than glucose and less diabetogenic. As in organic acid content there are no works available where the sugar contents of borojo are reported. However, the sugar contents in "exotic" fruits are widely studied. Thus, Alezandro et al. (2013) reported that the sugars found in jaboticaba (*Myrciaria cauliflora*) fruit were sucrose, glucose and fructose with values of 16.6, 17.0 and 21.4 g/100 g respectively. In a similar study Guo et al. (2015) analyzed the sugar contents of jujube (*Ziziphus jujuba*). These authors reported that the sugars found were sucrose, glucose and fructose with values of 19.0, 33.0 and 21.3 g/100 g respectively.

The nature and concentration of organic acids and sugars in the fruit depend on factors such as species, varieties and environmental conditions, including the climate, soil and irrigation practices (Marsh et al., 2003).

3.5. Volatile profile

It is well established that the aroma of fruits is highly dependent on the volatile fraction composition. Present in minor concentrations, volatile compounds are secondary metabolites of plants which greatly influence their sensorial quality. It is important to bear in mind that the volatile compounds were obtained using headspace solid phase micro-extraction (HS-SPME). This technique is better than simultaneous distillation-extraction (SDE) which can produce the formation of artefacts, mainly hydrolysis reactions which may lead to sugar degradation products, such as furfural.

When the volatile fraction of borojo was analyzed, a total of 21 compounds were identified. Table 3 shows the relative areas for the mean values of the identified compounds. The volatile fraction was distributed by different chemical classes: eight alcohols, eight esters, two ketones, two carboxylic acids and one monoterpen compound. The alcohols family represents the 68.78% of the total volatile compounds with 2-nonanol (53.52%), responsible for fruity and pulpy aroma, and 2-heptanol (6.36%) as the main components. Similarly, the esters represent the 15.88% of the total volatile compounds with benzyl acetate (6.96%) as principal compound. It is important to note that limonene (monoterpen compound) represents 4.20%. It widely demonstrates that this compound showed antioxidant, antimicrobial and anti-proliferative properties (Roberto et al., 2010). The compounds identified in borojo fruit were lower than that identified in other "exotic" fruit. Thus, Sousa Galvão et al. (2011) detected 246 compounds in umbu (*Spondias tuberosa*) fruits in which 80 were positively identified. The

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Table 2
Organic acids and sugar content of borojo (*Borojoa patinoi*) fruits.

Sugars (g/100 g sample)				Organic acids (mg/100 g sample)				
Maltose	Sucrose	Fructose	Glucose	Oxalic acid	Citric acid	Tartaric acid	Malic acid	Ascorbic acid
1.51 ± 0.07	0.61 ± 0.03	4.26 ± 0.14	1.35 ± 0.05	49.90 ± 0.48	484.68 ± 13.08[a]	1291.72 ± 12.01	732.25 ± 4.48	48.19 ± 0.23

(Mean ± SD) of five samples.

Table 3
Volatile compounds, expressed as area percentage, identified in borojo (*Borojoa patinoi*) fruit.

Family	Compound	RT	KI (Exp)	KI (LIT)	Peak area (% area)
Alcohols	Ethanol	5.72	543	–	2.98
	2-Pentanol	6.83	657	662	0.45
	1-Hexanol	9.50	872	869	2.65
	2-Heptanol	10.28	919	915	6.36
	1-Octanol	16.54	1074	1072	1.88
	2-Nonanol	17.74	1100	1098	53.52
	4-Decanol	22.33	1199	1203	0.50
	3-Nonen-1-ol	24.65	1247	1244	0.44
Esteres	Ethyl acetate	6.38	611	613	0.66
	Butyl acetate	8.29	803	810	0.68
	1-Methylethyl acetate	8.68	846	847	0.34
	Hexanoic acid methyl ester	10.90	934	941	0.97
	Ethyl hexanoate	13.35	994	997	2.52
	Methyl octanoate	18.63	1119	1110	1.28
	Benzyl acetate	20.86	1167	1165	6.96
	Ethyl octanoate	22.00	1192	1197	2.47
Acids	Acetic acid	6.23	595	600	2.74
	Hexanoic acid	12.52	973	976	5.56
Ketones	2-Heptanone	10.04	889	889	0.61
	2-Nonanone	17.27	1090	1091	2.23
Monoterpens	Limonene	14.97	1033	1030	4.2

RT: retention time; KI (Exp): Kovats Index experimental; KI (Lit): Kovats Index literature.

identified compounds were 34 alcohols, 17 esters, 16 ketones, 11 aromatic, 9 aldehydes, 8 terpenes, 7 furans and 6 sulfur compounds. In a similar work, Ong et al. (2008) identified in Jackfruit (*Artocarpus heterophyllus* L.) 37 compounds, including 20 esters, 5 alcohols, 9 aldehydes, 2 ketones and one ether.

3.6. Antimicrobial activity

Colombian people consume borojo shaken with water in proportion of about 30% (w/v); for this reason, in our study, we use this concentration to perform the antimicrobial activity. Borojo aqueous extract (BAE) was tested against pathogenic and deteriorative bacteria isolated from meat products, in particular *L. monocytogenes* (7 strains), *S. aureus* (5 strains) *S. thyphymurium* (8 strains) *S. enteritidis* (2 strains) and *B. thermosphacta* (4 strains).

In general, all the strains were inhibited by the BAE showing inhibition halos ranging between 10.3 ± 0.7 and 22.4 ± 0.9 mm. The most sensible strains were *S. enteritidis* SE4406 (22.4 ± 0.9 mm) followed by *S. typhimurium* SE48 (15.32 ± 0.5 mm) and Gram-positive bacteria (*L. monocytogenes*, *S. aureus* and *B. thermosphacta*) (Table 4). In literature, very few reports evidenced the antimicrobial activity of borojo against pathogenic bacteria; the only data are reported by Sotelo et al. (2010) which evidenced a major sensibility of *S. aureus* ATCC 460716 compared with *E. coli* ATCC 25922.

The pH of the BE*w* used in this study to determine the antimicrobial activity was 2.93, and this condition substantially affects the growth of the species tested. Thus, in order to evidence the contribution of pH to the antimicrobial activity, the pH of the BAE was raised up to 6.0. The comparison of the inhibition diameters of both solutions evidenced a slight but significant reduction ($P < 0.05$) of the antimicrobial activity with all strains tested (Table 4). As reported above, the organic acids, mainly tartaric acid

Table 4
Spectrum of antimicrobial activity of borojo (*Borojoa patinoi*) solution.

Strains	Diameter of inhibition (mm)	
	Borojo solution (pH = 2.9)	Borojo solution (pH = 6.0)
Br. thermosphacta B01	13.2 ± 0.3^{aA}	10.3 ± 0.3^{aB}
Br. thermosphacta B02	13.0 ± 0.1^{aA}	10.0 ± 0.1^{aB}
Br. thermosphacta B03	12.1 ± 0.1^{bA}	8.1 ± 0.2^{bB}
Br. thermosphacta B04	14.4 ± 0.6^{cA}	12.0 ± 0.5^{cB}
L. monocytogenes LM15	13.2 ± 0.2^{aA}	8.1 ± 0.2^{bB}
L. monocytogenes LM56	13.0 ± 0.2^{aA}	11.4 ± 0.3^{cB}
L. monocytogenes LM49	13.7 ± 0.4^{cA}	11.2 ± 0.2^{cB}
L. monocytogenes LM16	13.2 ± 0.1^{aA}	12.5 ± 0.3^{cB}
L. monocytogenes ATCC 19144	16.3 ± 0.8^{dA}	13.0 ± 0.5^{eB}
S. aureus STA59393	13.4 ± 0.4^{aA}	11.1 ± 0.3^{dB}
S. aureus STA39533	13.1 ± 0.2^{aA}	12.7 ± 0.3^{cB}
S. aureus CF025	14.3 ± 0.5^{cA}	13.2 ± 0.3^{dB}
S. aureus CF032	15.1 ± 0.2^{aA}	12.6 ± 0.3^{dB}
S. aureus CP2801	13.3 ± 0.5^{aA}	11.1 ± 0.3^{dB}
S. typhimurium S6	12.3 ± 0.4^{aA}	10.5 ± 0.3^{aB}
S. typhimurium S3	12.1 ± 0.2^{aA}	10.1 ± 0.2^{aB}
S. typhimurium S5	12.1 ± 0.1^{aA}	10.3 ± 0.2^{aB}
S. typhimurium S4	14.3 ± 0.3^{bA}	12.1 ± 0.6^{bB}
S. typhimurium S1	12.3 ± 0.5^{bA}	10.7 ± 0.4^{aB}
S. typhimurium ST56	13.4 ± 0.5^{cA}	10.4 ± 0.5^{aB}
S. typhimurium ST37	12.2 ± 0.3^{aA}	10.0 ± 0.2^{aB}
S. typhimurium ST44	15.3 ± 0.5^{bA}	12.2 ± 0.3^{bB}
S. enteritidis SE4406	22.4 ± 0.9^{dA}	16.7 ± 0.8^{cB}
S. enteritidis SE2	10.3 ± 0.7^{eA}	9.4 ± 0.2^{dA}

Mean and standard deviation of five repetitions.
Different superscript letters in the same column mean significant differences between the strains ($p < 0.05$).
Different capital letters in the same line mean significant differences between the treatments ($p < 0.05$).

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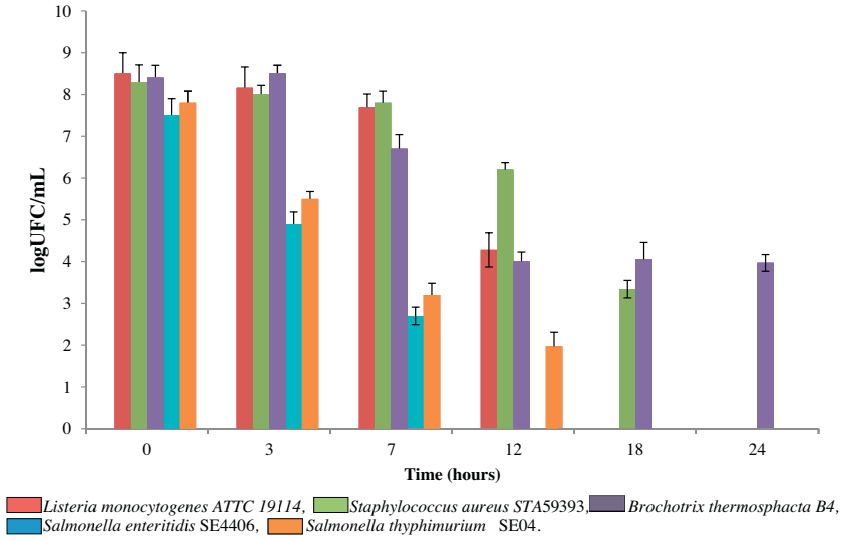

Fig. 2. Time kill kinetics for borojo (*Borojoa patinoi*) solution against selected bacteria strains at 30 °C.

and malic acid, were present in high quantities in the BE*w*. It is well known that in the non-dissociated form, acids enter into the cell, where they dissociate, lowering the cytoplasmic pH and disrupting the normal physiology of pH-sensitive bacteria, including *L. monocytogenes* and *Salmonella* spp. Organic acids in the anionic form cannot pass through the cell membrane, and therefore they accumulate within the cell, increasing osmotic pressure and disrupting some metabolic functions (Paparella et al., 2013). The effect of organic acids against Gram-negative bacteria has been observed by Helander and Mattila-Sandholm (2000) who reported that citric acid, an organic acid abundant in berries, destabilize and permeabilize the bacterial membrane, moreover it was reported that malic acid, lactic acid, benzoic acid and sorbic acid efficiently destabilized and disintegrated the outer membrane of *Salmonella* spp (Alakomi et al., 2007). With the results obtained here it can be hypothesized that the antibacterial activity displayed by BS could be mostly linked by multiple mechanisms and synergies because it contains various compounds present in the in their chemical composition. In fact, it is well known that secondary metabolites such as phenolic compounds, terpenes, tannins saponines and alkaloids contributed to the antimicrobial activity of the vegetal extract (Reuben et al., 2008).

The results of antibacterial activity of BE_w obtained by disc diffusion method were confirmed by TKK. This parameter provides a greater information of the cell eradication potential of antibacterial agents; thus, for the most sensitive strains, TKK assays were performed. As shown in Fig. 2, the kinetics of inactivation monitored over 24 h confirmed the activity of BAE against the strains tested; however, comparing the antibacterial activity for these most sensitive strains, it is possible to evidence that *S. enteritidis* SE4406 and *S. typhimurium* SE04 showed the major sensibility. In fact, they showed a significant reduction ($P > 0.05$) in cell counts of about 2.5 log CFU/mL in the first 3 h of exposure indicative of a bacteriostatic effect; a continued exposure determined a reduced to a level consistent with bactericidal effect that was achieve after 7 and 13 h for *S. enteritidis* SE4406 and *S. typhimurium* SE04 respectively. On the other hand, the bactericidal effect towards *L. monocytogenes* ATTC19114 and *S. aureus* STA59393 was achieved within first 18 and 24 h respectively. On the contrary during the experimental time BS had a bacteriostatic effect on *B. thermosphacta* B4 strain.

As previously reported by Paparella et al. (2013), plant extracts are characterized by a very complex and rich composition. Therefore, several mechanisms, acting on specific targets simultaneously, have been proposed to explain their antimicrobial action. Indeed, particular attention must be played to phenolic compounds, as they seem to be responsible of antimicrobial action of plant extracts. The sensibility of *L. monocytogenes* to phytochemical compounds has been reported by other authors (Cornu et al., 2006). In particular, antilisterial activity of different plant polyphenols is previously referred only in a few studies. Recently the antilisteria action of the phenolic extracts of lowbush blueberries has been evidenced (Lacombe et al., 2012). Moreover, hydroxytyrosol, carnosic acid and epicatechin had an antilisteria effect which occurs in a concentration-dependent manner (Bubonja-Sonje et al., 2011). Many reports suggested that Gram-positive bacteria are more sensible than Gram-negative ones to polyphenols derived from fruits. This higher resistance could be attributed to the presence of an additional external membrane surrounding the cell wall in Gram-negative bacteria, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Burt, 2004). A recently study realized by Cui et al. (2012) using (−)-Epigallocatechin-3-gallate (EGCG), a main constituent of tea catechins, suggests that the damages on the cell wall of Gram-positive bacteria are caused by its direct binding to the peptidoglycan layer, whereas its damage to the cell walls of Gram-negative bacteria are induced mainly by H_2O_2 production generated from EGCG in phosphate buffer at neutral pH. It should be noted that chlorogenic and ferulic acids were the most abundant polyphenols present in the BE*w* in our study. Ferulic acid has been reported to have selective ability to inhibit *L. monocytogenes* and a relative ineffectiveness at concentration of 1500 ppm against *Escherichia coli* O157:H7, *Salmonella enterica*, and *Pseudomonas fluorescens* (Takahashi et al., 2013). Also chlorogenic acid showed good antimicrobial activity against *E. coli*, *B. subtilis* and *S. Aureus* (Zhao et al., 2010). In this context we may presume that part of the antimicrobial activity of BAE might be related to synergy of phenolic and organic acids recorded. However, the major sensibilities showed by *S. enteritidis* SE4406 indicate that other water-soluble compounds present in borojo fruit might give rise to the antibacterial activity. In fact, as stated above the most important volatile group of borojo was represented by alcohols and in particular 2-nonanol which was present in high amounts. Mekni et al. (2013) reported that the volatile fractions coming from *Punica granatum* L. flowers showed a moderate effect against *E. coli* and *P. aeruginosa*. Thus it is difficult to attribute the activity of a complex mixture as is the BAE to a single or particular constituent. Bassole

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et al. (2003) suggested that the existence of some antimicrobial constituents combined with other minor constitutes might be involved in improving overall antimicrobial activity of volatile fractions. So, it is possible to hypothesize that antimicrobial effects of borojo fruit may be attributed to the combined action of various phytochemicals which cause damage to cell membranes, causing leakage of cellular materials and ultimately the bacterial death.

4. Conclusions

The present study represents a contribution to the chemical characterization of borojo fruit. Their chemical characteristics induce in general a considerable antioxidant activity and strong bactericidal action against *Salmonella* spp., *L. monocytogenes* and *S. aureus*, which are some of the most common sources of food-borne diseases, although it presented some strain specific activity that assumes the role of different chemical compounds present in the extracts.

Our findings suggested that borojo has great potential to be used as a natural food biopreservative and as an ingredient to produce functional foods. However more investigations are needed related to chemical constituents with antimicrobial activities to better understand their molecular targets and mechanisms of action.

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