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Red-jambo *(Syzygium malaccense)*: Bioactive compounds in fruits and leaves

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ABSTRACT

Syzygium malaccense is poorly studied regarding the bioactive compounds of fruits and leaves. The aim of this study was to determine proximate composition, phenolic compounds, carotenoids, and antioxidant capacity of the fruit parts and leaves of *S. malaccense*. The samples were extracted with different solvents in order to analyze phenolic compounds content (Folin-Ciocalteau and HPLC-DAD/FLD), flavonoids (reaction with $AlCl_3$ and HPLC-DAD/FLD), anthocyanins (differential pH and HPLC-DAD, UPLC-ESI-MS/MS), total carotenoids (colorimetric method) and antioxidant capacity (hydrophilic and lipophilic-ORAC, FRAP, DPPH). The pulp demonstrated high amounts of soluble fibers and reducing sugars. The peel, seeds and leaves of *S. malaccense* showed great contents of phenolic compounds, flavonoids and carotenoids as well as antioxidant capacity. The anthocyanins found in the fruit were cyanidin-3-*O*-glucoside, cyanidin-3,5-*O*-diglucoside, and peonidin-3-*O*-glucoside. Polar bioactive compounds showed strong correlation to hydrophilic antioxidant capacity, while carotenoids did not correlate lipophilic-ORAC.

1. Introduction

Several berries have shown great scientific interest due to antioxidant power, antitumoral and anti-inflammatory effects (Nile & Park, 2014; Paredes-López, Cervantes-Ceja, Vigna-Pérez, & Hernández-Pérez, 2010). *Syzygium malaccense* (syn. *Eugenia malaccensis, Jambos malaccensis*) belongs to the *Myrtaceae* family and is an original plant from Malaysia, known as Malay apple. Although, the plant was widespread throughout tropical regions, where was also named as pomerac, mountain-apple or red-jambo (Morton, 1987).

The tree reaches 12–15 m height, shows straight trunk and pyramidal canopy. The red-jambo tree could be used as ornamental plant due to the beauty of its fallen flowers, and offers berry fruits two times per year: February–March and October–November. The fruits are pear-shaped, succulent, containing a single seed, and thin, smooth, waxy peel varying from the rose, crimson, and dark red colors, depending on the stage of maturation or harvest conditions. The pulp is whitish and juicy with acid flavor, similar to apples (Morton, 1987). The aroma of red-jambo is similar to a mixture of roses and herbaceous plants (Pino, Marbot, Rosado, & Vazquez, 2004). The fruits are eaten fresh or in form of handmade products. Although, much of the fruit is wasted during harvest time due to high production, perishability and lack of technological feasibility information for the use by industries.

The scientific literature reports antioxidant capacity of the edible part of red-jambo fruits (Lako et al., 2007; Reynertson, Yang, Jiang, Basile, & Kennelly, 2008), anti-inflammatory and antioxidant effects of leaves use (Andersson Dunstan et al., 1997; Arumugam, Manaharan, Heng, Kuppusamy, & Palanisamy, 2014), cytotoxic power of leaves (Savitha, Padmavathy, & Sundhararajan, 2011), and glycemia/cholesterolemia-lowering effects of the trunk bark

Abbreviations: FPP, fresh pulp + peel; PP, pulp + peel; TA, titratable acidity; SS, soluble solids; MetOH, 80% methanol extract; H-MetOH, hydrolyzed 80% methanol extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity; H-ORAC, hydrophilic ORAC; L-ORAC, lipophilic ORAC; T-ORAC, total ORAC; BHT, 2,6-di-tert-butyl-4-methylphenol; GAE, gallic acid equivalents; CE, catechin equivalents; TE, trolox equivalents; FLD, fluorescence detector; PCA, principal component analysis.

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extract (Bairy, Sharma, & Shalini, 2005). Thus, each tissue of the plant has different chemical characteristics that encourage research to explore their functional effects in separated way, since this data is previously rare in literature.

The aim of this work was to explore different tissues of *S. malaccense* (pulp, peel, seed and leaf) in relation to nutrients composition and phytochemicals with bioactive function, such as phenolic compounds and carotenoids, and to determine antioxidant capacity of the different plant tissues.

2. Material and methods

2.1. Standards

Benzoic acid standard was purchased from Chem Service (West Chester, USA). Kaempferol-3-*O*-glucoside, (+)-catechin, cyanidin-3,5-*O*-diglucoside-chloride (cyanin chloride), cyanidin-3-*O*-glucoside-chloride (kuromanin chloride), (−)-epicatechin, (−)-epicatechin gallate, isorhamnetin-3-*O*-glucoside, peonidin-3-*O*-glucoside chloride, procyanidin A2, procyanidin B1, procyanidin B2, quercetin (dihydrate), isoquercitrin, and rutin standards were obtained from Extrasynthese (Genay, France). *p*-Coumaric acid was purchased from Sigma (UK).

2.2. Samples

Ripe red-jambo fruits were harvested in Araçatuba-SP in February 2013 and purchased from a local market in Campinas-SP, Brazil.

Physical-chemical determinations: Ten ripe fresh fruits were randomly chosen (Suppl. 1), cleaned and pulp, peel, pulp + peel (PP) and seeds were separated and weighed. Titratable acidity (TA), soluble solids and pH were determined in the edible part (fresh pulp + peel = FPP). The TA was determined by titration with standardized 0.01 N NaOH and the results were expressed as grams of citric acid. Soluble solids (SS) were measured in a manual refractometer. The pH was evaluated using a digital pH meter (Ion Meter 450).

The leaves were collected in the main campus of the University of Campinas and cleaned. The leaves and the parts of the fruits were dried in a freeze-dryer (LP1010, Liobras, São Carlos, São Paulo, Brazil) at a range from −40 to 25 °C, 300 μm Hg for 95 h, crushed, homogenized and frozen at −18 ± 5 °C.

2.3. Macronutrient determinations

Total nitrogen was determined using a NDA 701 Dumas Nitrogen analyzer (VelpScientifica, Usmate, Italy). Moisture and ash were analyzed according to standard methods (AOAC., 2002) and lipids according to Bligh and Dyer (Bligh & Dyer, 1959). Carbohydrates were calculated by difference. Total and insoluble dietary fibers were quantified by the enzymatic–gravimetric method (AOAC., 2002). Soluble fibers were calculated by difference.

In order to determine reducing sugars, a 70% ethanol solution was added to the samples and allowed to react during 60 min at 100 °C. After ethanol evaporation, the extracts were filled with water and then analyzed according to the Somogyi-Nelson method, using a Synergy HT, Biotek microplate reader (Winooski, USA), with readings set at 520 nm (Nelson, 1944).

2.4. Bioactive compounds and antioxidant capacity measurements

All the absorbance and fluorescence readings for the analyses were determined in a Synergy HT, Biotek microplate reader (Winooski, USA) with Gen5™ 2.0 data analysis software.

2.4.1. Sample treatments

Extracts (40 g L^{-1} for freeze-dried samples and 120 g L^{-1} for FPP) were made in duplicate as follows:

80% Methanol extract: The samples were extracted with 80% methanol (MetOH:H_2O, v:v) at 37 °C for 3 h in a shaking water bath, centrifuged at 2000× *g*, for 10 min, and stored in amber flasks at 4–8 °C (Batista, Ferrari, da Cunhada SilvaCazarin, & Correa, 2016).

Hydrolyzed 80% methanol extract: A HCl and BHT (2,6-di-tert-butyl-4-methylphenol) solutions were added to the aforementioned methanol extract to result in a final concentration of 1.2 mol L^{-1} HCl, 0.26 g L^{-1} BHT in 50% methanol (MetOH:H_2O, v:v) in the extract. In order to complete the hydrolysis, the extracts were allowed to react in water bath at 90 °C for 30 min, with refrigerated reflux condenser.

Lipophilic extract: The samples were extracted by successive maceration with hexane at room temperature to obtain a non-polar extract. After solvent evaporation, samples were resuspended with acetone (Prior et al., 2003).

All extracts were stored at 4 °C until analyzed and new extracts were made each 5 days.

2.4.2. Bioactive compounds

Total phenolic compounds or Folin-Ciocalteau reagent reducing substances method: Folin-Ciocalteau reagent and sodium carbonate were added to water-diluted extract, and after 2 h in the dark at room temperature, the absorbance of samples and standard curve was read at 725 nm. Gallic acid was used as standard and results were expressed as gallic acid equivalents (mg GAE) (Batista et al., 2016).

Total flavonoids: Solutions, such as 5% sodium nitrite, 10% aluminum chloride and 1 mol L^{-1} sodium hydroxide were added to water-diluted extracts (Zhishen, Mengcheng, & Jianming, 1999). A precipitation was observed after the addition of NaOH solution to the leaf extract, and because of that the mix was centrifuged at 2000× *g*, 10 min. A calibration curve was made using (+)-catechin. The samples and standard were read at 510 nm. The results were expressed as catechin equivalents (mg CE).

Monomeric anthocyanins method: The extracts were diluted using 0.025 mol L^{-1} potassium chloride buffer (pH 1.0) according to sample absorbance (0.4–0.6), and in 0.4 mol L^{-1} sodium acetate buffer (pH 4.5) using the same proportions (1:2, 1:6 and 1:15 dilution factors for FPP, PP and peel, respectively). After addition of 250 μL in the microplate, the absorbance was read at 520 and 700 nm (Wrolstad, 1976). The absorbance (A) was calculated using Equation (1):

$$A = [(A_{520\ nm} - A_{700\ nm})pH = 1.0] - [(A_{520\ nm} - A_{700\ nm})pH = 4.5] \quad (1)$$

The anthocyanin content (mg 100 g^{-1}) was calculated as cyanidin-3-*O*-glucoside (PM = 449.2) using Equation (2):

$$C\left(mgC3G\ 100g^{-1}\right) = A.MW.DF/\xi.L \quad (2)$$

where C = concentration, C3G = cyanidin-3-*O*-glucoside, ξ = molar absorptivity (26,900 mol L^{-1}), L = pathlength (cm), MW = molecular weight and DF = dilution factor.

HPLC analysis: The analyses of the fruit extracts were performed using a HPLC system Waters e2695 Separation Module Alliance equipped with a quaternary solvent pump and an automatic injector. For the phenolic compounds determination, a diode array detector (DAD) Waters model 2998 and a fluorescence detector (FLD) Waters model 2475 were employed. Acquisition and

processing of data were carried out using the Waters Empower™ 2 software (Milford, USA).

The extracts were filtered through 0.45 μm nylon membranes (Allcrom-Phenomenex, USA). The injection volume was 10 μL. The Gemini NX C-18 column (150 mm × 4.6 mm × 3 μm) (Phenomenex, USA) was maintained at 40 °C. The mobile phase consisted of a gradient mixture of solvent A (0.85% aqueous phosphoric acid solution) and solvent B (acetonitrile), with 0.5 mL min^{-1} flow-rate. The gradient was started with 100% solvent A and adjusted for 93% A and 7% B at 10 min; 90% A and 10% B at 20 min; 88% A and 12% B at 30 min; 77% A and 23% B at 40 min; 65% of solvent A and 35% of solvent B at 45 min; and 100% B at 55 min.

Fluorescence detector was used at 320 nm emission for identification of the following compounds: (+)-catechin, procyanidin B2, procyanidin A2 and (−)-epicatechin. The DAD was set in four wavelengths: 280 nm for identification of gallic acid and tannins: (−)-epicatechin gallate and procyanidin B1; 320 nm for phenolics acids; 360 nm for flavonols and 520 nm for anthocyanins.

UPLC-ESI-MS/MS analysis: After successive extractions with 1% aqueous formic acid, the anthocyanins from the freeze-dried peel were extracted using a solid-phase - manifold apparatus and methanol, water, ethyl acetate and 1% formic acid in methanol as solvents, concentrated under gas nitrogen atmosphere, resuspended in water, filtered through 0.22 μm syringes and analyzed by ultra-high performance chromatography − mass spectrometry (UPLC−MS). An Acquity UPLC system (Waters, Milford, MA, USA) was used coupled with UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm particle size) at 30 °C and 3 μL of the extract were injected. Mobile phase was used as follows: a gradient of (A) deionized purified water with 1% aqueous formic acid and (B) acetonitrile starting with 5% B and ramping to 100% B at 8 min, maintained until 8.5 min, then returning to initial conditions and stabilizing by 10 min. Detection in positive ion modes was achieved on an Acquity TQD mass spectrometer (Micromass Waters, Milford, MA, USA) with capillary − 3000 V, Cone − 30 V, source temperature 150 °C; desolvation temperature 350 °C.

The phenolic compounds in the leaves of *S. malaccense* were performed using a HPLC-DAD method as described previously (Batista et al., 2014).

Total carotenoids method: The lipophilic extract was shaken in an Erlenmeyer flask containing isopropanol and hexane, transferred to a separation funnel and gently washed with water, 3 times. The hexane extract was filtered in sodium sulfate and filled with hexane (Higby, 1962). The samples and blank (hexane) were read at 450 nm in the microplate reader. The calculations were performed as follows (Equation (3)):

$$C\left(mg\ 100g^{-1}\right) = (A.100)/(250.L.W) \quad (3)$$

where C = concentration, A = absorbance, L = wells pathlength (cm), W = sample weight mL^{-1} final solution.

In order to eliminate possible interferences of chlorophyll content, the same extract used on carotenoid measurements was read at 649 and 665 nm and proper calculations were made (Total chlorophyll: (6.45. A_{665}) + (17.72. A_{649})) (Wellburn, 1994).

2.4.3. Antioxidant capacity

Antioxidant capacity was assessed using FRAP, DPPH and ORAC methods as described previously (Batista et al., 2016), with some adaptations:

FRAP method: Samples and trolox standard curve were read at 595 nm. The results were expressed as trolox equivalents (μm TE).

DPPH-IC_{50}: The reading time of the extracts in DPPH analysis were 15 min for leaves, seeds and peel, 60 min for PP, 3 h for pulp and 24 h for FPP, respectively. The concentration of samples required in order to reduce the 50% DPPH radical (IC_{50}) was calculated by linear regression.

ORAC: The H-ORAC (hydrophilic oxygen radical absorbance capacity) and L-ORAC (lipophilic-ORAC) tests were carried out according to previous descriptions (Batista et al., 2016). In addition, the total-ORAC (T-ORAC) was assessed by the sum of H-ORAC values of the MetOH extracts and L-ORAC values.

2.5. Statistics

Due to peculiarities in chemical composition, no statistical tests were used to assess differences among the different tissues of *S. malaccense*. The comparison between the data of methanol and hydrolyzed extracts was evaluated using Student T-test, considering $P < 0.05$. The correlations were made considering Pearson's coefficient ($P < 0.05$). GraphPad Prism 5.0 software (GraphPad, Inc. La Jolla, CA, USA) was used for the aforementioned analyses.

The Principal Component Analysis (PCA) was made using the SAS software, version 9.4 (SAS Institute Inc., Cary, USA).

All results were expressed as g or 100 g of wet weight of freeze-dried samples (PP, peel, pulp, leaves and seeds), as well as wet weight of fresh pulp + peel.

3. Results

Nutrients, bioactive compounds and antioxidant capacity of *S. malaccense* fruit parts and leaves were explored in this study. The PP, fresh and dried, was also studied since it is the usual eaten or most studied form. Phenolic compounds and lipophilic bioactive compounds were related with great antioxidant power in all tissues of *S. malaccense*, as detailed below.

The whole fresh fruit weighed 75.86 ± 16.26 g. The proportion of FPP in the fruits was 64.58 ± 20.85%, showing high variation in the fresh pulp part (47.21 ± 20.95%), but not in the fresh peel (17.37 ± 1.03%), and seeds (18.94 ± 2.48%) (Suppl. 1). The pH of the FPP was 3.72 ± 0.01, the SS 4.67 ± 0.2% and TA reached 0.628 ± 0.01 g citric acid 100 g^{-1} wet weight (SS/TA ratio = 7.37 ± 0.3).

S. malaccense contains high amounts of water and reducing sugars (Table 1). The peel of the red-jambo concentrated more protein (total N) and lipids, but less reducing sugars than the pulp. The dietary fiber content was higher in the peel, although the pulp has shown higher soluble fiber amounts (Table 1).

The level of phenolic compounds varied depending on the plant tissue type and different sample treatment methods. The leaves, peel and seeds showed the highest amounts of total phenolic compounds and flavonoids (Table 2). The acid hydrolysis of MetOH extracts allowed the release of phenolic monomers, increasing the quantification of total phenolic compounds found in the PP (2.85 times) and leaves (1.67 times) (Table 2). Phenolic compound levels were significantly correlated with hydrophilic antioxidant power of *S. malaccense* (phenolic compounds × ORAC: $r = 0.9841$, $P < 0.0001$; flavonoids × ORAC: $r = 0.9876$, $P < 0.0001$). The Folin-Ciocalteau method was also performed on the BHT solution used for the acid hydrolysis at the same conditions as the samples, and it was found a value of 26.98 ± 0.73 mg GAE 100 g^{-1} for the final solution.

The peel of red-jambo also concentrated the monomeric anthocyanins from the edible part (Table 2), which corroborates its higher hydrophilic antioxidant capacity (Table 3). In addition, due to the low pH, methods for quantification of total flavonoids and monomeric anthocyanins in the hydrolyzed extracts were not done.

The hydrolyzed extracts of peel and leaves showed similar FRAP values compared to the MetOH extracts (Fig 1). Indeed, the phenolic compounds content was not increased in hydrolyzed peel

Table 1
Proximate composition (%) of *S. malaccense* fruit parts.[a]

Composition	FPP	Pulp[b]	PP[b]	Peel[b]
Moisture	93.09 ± 0.24	4.81 ± 0.13	4.83 ± 0.22	4.27 ± 0.06
Protein (N × 6.25)	0.45 ± 0.014	3.10 ± 0.13	5.23 ± 0.07	5.57 ± 0.10
Lipids	0.20 ± 0.008	1.24 ± 0.05	2.62 ± 0.04	5.92 ± 0.12
Ash	0.31 ± 0.006	3.39 ± 0.083	3.85 ± 0.12	5.06 ± 0.131
Total carbohydrates	6.19 ± 0.51	87.65 ± 0.32	83.73 ± 0.16	78.59 ± 0.19
Reducing sugars	2.25 ± 0.04	64.98 ± 3.69	49.55 ± 0.91	48.42 ± 2.09
Total dietary fibers	1.54 ± 0.02	28.50 ± 0.89	33.67 ± 0.52	36.16 ± 0.006
Insoluble dietary fibers	1.28 ± 0.03	20.94 ± 1.55	27.92 ± 0.006	32.78 ± 0.46
Soluble dietary fibers	0.25 ± 0.01	8.00 ± 0.54	5.62 ± 0.22	3.53 ± 0.17

[a] Expressed as wet weight of samples.
[b] Freeze-dried samples. PP: pulp + peel, FPP: fresh PP.

Table 2
Bioactive compounds in *S. malaccense* fruit and leaves analyzed by colorimetric methods.

	Extracted samples	mg 100g^{-1}[a]
Total polyphenols	MetOH FPP	14.81 ± 1.14
	MetOH Pulp	28.40 ± 1.92
	MetOH PP	158.26 ± 4.23***
	H-MetOH PP	232.18 ± 14.80
	MetOH Peel	392.92 ± 6.38
	H-MetOH Peel	394.20 ± 33.42
	MetOH Leaves	5377.08 ± 129.06***
	H-MetOH Leaves	8873.03 ± 407.71
	MetOH Seed	1063.81 ± 68.45
Total flavonoids	MetOH FPP	12.86 ± 0.41
	MetOH Pulp	13.12 ± 1.029
	MetOH PP	81.89 ± 5.52
	MetOH Peel	267.84 ± 9.43
	MetOH Leaves	810.22 ± 46.75
	MetOH Seed	154.37 ± 25.74
Monomeric anthocyanins	MetOH FPP	12.90 ± 0.63
	MetOH PP	126.95 ± 0.42
	MetOH Peel	424.82 ± 0.42
Total carotenoids	Non-polar Pulp	0.015 ± 0.001
	Non-polar PP	0.046 ± 0.007
	Non-polar Peel	0.158 ± 0.011
	Non-polar Leaves	3.93 ± 0.55[b]
	Non-polar Seed	0.16 ± 0.008

FPP: fresh pulp + peel, PP: pulp + peel.
* Indicates statistical differences from the respective hydrolyzed extracts according to Student t-test (***$P < 0.001$).
[a] Expressed as wet weight of samples.
[b] Value discounted of chlorophyll amount in the carotenoid extract (6.53 ± 0.27 mg 100g^{-1}).

extract like as in PP, which corroborated the FRAP values (FRAP × phenolic compounds: $P < 0.0001$, $r = 0.9696$). Although, hydrolysis released a significant portion of radical scavenging capacity according to the ORAC values, since it was around 3 times higher in all the H-MetOH extracts (Table 3).

The samples showed good linearity ($R^2 > 0.95$) in the concentration ranges studied in DPPH-IC_{50} and H-ORAC (Table 3). Regarding the DPPH-IC_{50} values, the antioxidant capacity was higher in the following crescent order FPP < Pulp < PP < Peel < Seeds < Leaves (Table 3).

The non-polar extract of the peels showed higher L-ORAC value, followed by seeds and leaves, which had approximately the same value (Table 3). Although, due to the H-ORAC, T-ORAC values were higher in the dried leaves (528.93 ± 16.44 μmol TE g^{-1}), followed by the peel (135.47 ± 9.47 μmol TE g^{-1}), seeds (108.78 ± 7.99 μmol TE g^{-1}), PP (70.16 ± 3.49 μmol TE g^{-1}), and pulp (14.20 ± 0.14 μmol TE g^{-1}).

Total carotenoids were high in leaves (Table 2), but the data was not correlated with the lipophilic-ORAC, since the highest L-ORAC value was found in the peel and not in the leaves as in the carotenoids assay.

Regarding the phenolic compounds profile, tannin amounts were smaller after hydrolysis of red-jambo fruit extracts, mainly the (−)-epicatechin gallate (non-detected), procyanidins B1 (non-detected) and A2 contents. The MetOH PP extract showed higher contents of (+)-catechin and (−)-epicatechin in comparison to the peel. However, the amount of such compounds was increased in the H-MetOH peel extracts (Table 4).

Phenolic acids were also increased with the hydrolysis of the PP extract. In the peel, benzoic acid was detected only in MetOH and p-coumaric only in H-MetOH (Table 4).

In relation to flavonols, isorhamnetin, quercetin and isoquercitrin, the amounts were higher in peel of red-jambo compared to the PP. Rutin and kaempferol-3-*O*-glucoside amounts were higher in H-MetOH PP extract. Both hydrolyzed extracts increased the quantification of the aglycone quercetin, probably because of hydrolysis of its glycosylated compounds (Table 4).

In addition, linear combinations among the phenolic compounds found in the extracts of PP and peel of red-jambo were separated out in different clusters by PCA (Fig. 2). The non-hydrolyzed peel extract could be grouped by the condensed tannins ((−)-epicatechin gallate and procyanidin B1), benzoic acid, isorhamnetin-3-*O*-glucoside and isoquercitrin contents, and the PP by procyanidin A2. In the other hand, the H-MetOH PP was characterized for the content of kaempferol-3-*O*-glucoside and rutin, corroborating aforementioned results; but the H-MetOH peel was correlated with the amounts of p-coumaric, procyanidin B2, (+)-catechin, (−)-epicatechin, quercetin and anthocyanins (Fig. 2).

Results showed that anthocyanins were the major class of phenolic compounds in the edible portion of red-jambo, representing more 75% of the total amount determined by HPLC (Table 4). Five anthocyanins were found in red-jambo peel, but two minor ones could not be identified. The three anthocyanins identified by UPLC-MS analysis in the positive ion mode were: Cyanidin-3,5-*O*-diglucoside ([M+] *m/z* 611, MS/MS fragment ions *m/z* 449, 287); cyanidin-3-*O*-glucoside ([M+] *m/z* 449, MS/MS fragment ions *m/z* 287), and peonidin + hexose ([M+] *m/z* 463, MS/MS fragment ions *m/z* 301). However, using HPLC-DAD and comparing retention time and elution order with identical standards, the hexose of peonidin was confirmed to be a 3-*O*-glucoside (Table 4). Cyanidin-3-*O*-glucoside was the most abundant anthocyanin quantified by HPLC-DAD, followed by cyanidin-3,5-*O*-diglucoside. In general, the hydrolysis increased quantification of anthocyanins in the fruit, most of them due their stability in acid conditions (Table 4, Fig. 2).

The H-MetOH extracts of the leaves of *S. malaccense* showed 464.42 ± 27.54 mg 100 g^{-1}, 377.91 ± 32.34 mg 100 g^{-1}, and 508.05 ± 10.40 mg 100 g^{-1} of (+)-catechin, (−)-epicatechin and quercetin, respectively.

Table 3
DPPH IC_{50}, H- and L-ORAC values and linearity of *S. malaccense* fruit parts and leaves.

Extracts		Mean ± SD[a]	Conc[b] (g L^{-1})	Slope	Intercept	R^2
DPPH IC_{50} (g L^{-1})	MetOH FPP	674.75 ± 40.03	100–750	0.0361	25.764	0.9740
	MetOH Pulp	61.36 ± 0.93	21–73	0.5933	13.587	0.9910
	MetOH PP	35.95 ± 0.49	5.0–40	0.9888	14.453	0.9904
	MetOH Peel	29.88 ± 0.52	5.0–40	1.1761	14.873	0.9869
	MetOH Leaves	0.67 ± 0.01	0.2–1.0	50.329	16.981	0.9581
	MetOH Seed	2.47 ± 0.05	1.0–4.0	14.8315	13.352	0.9921
H-ORAC (μmol TE g^{-1})	MetOH FPP	7.50 ± 0.46	1.0–5.5	5.146	3.272	0.9937
	MetOH Pulp	13.62 ± 0.11	2.0–5.0	7.732	5.7611	0.9912
	MetOH PP	59.99 ± 5.93***	0.3–0.9	46.406	−0.159	0.9957
	H-MetOH PP	168.53 ± 42.26	0.05–0.3	134.19	1.9084	0.9939
	MetOH Peel	108.75 ± 13.01*	0.1–0.6	93.566	−0.3837	0.9958
	H-MetOH Peel	362.52 ± 42.26	0.025–0.125	174.51	1.3157	0.9959
	MetOH Leaves	502.28 ± 33.88***	0.01–0.15	263.42	9.294	0.9975
	H-MetOH Leaves	1374.419 ± 125.06	0.015–0.035	825.9	6.3483	0.9651
	MetOH Seed	88.31 ± 8.79	1.6–8.0	7.024	1.5221	0.9980
L-ORAC (μmol TE g^{-1})	Non-polar Pulp	0.55 ± 0.031	2.0–5.0	$y = -0.0008x^2 + 0.1199x - 0.0563$		0.829
	Non-polar PP	10.50 ± 0.10	5.0–20.0	$y = -0.0025x^2 + 0.1617x - 11.663$		0.938
	Non-polar Peel	26.47 ± 1.57	2.8–8.0	$y = -0.3118x^2 + 4.3886x - 1.9594$		0.951
	Non-polar Leaves	12.88 ± 0.27	2.8–13.3	$y = -0.1501x^2 + 3.2613x - 2.978$		0.991
	Non-polar Seed	13.91 ± 0.40	2.8–13.3	$y = 1.4773x + 1.9641$		0.969

FPP: fresh pulp + peel, PP: pulp + peel. [a] Expressed as wet weight of samples. [b]Concentration ranges. *Indicates statistical differences from the respective hydrolyzed extracts according to Student t-test (*$P < 0.05$; ***$P < 0.001$).

4. Discussion

This study demonstrated that *S. malaccense* fruits and leaves have bioactive compounds with antioxidant capacity, such as phenolic compounds, carotenoids, as well as high amounts of dietary fibers.

Dietary fibers are associated with control of metabolic diseases, like obesity, diabetes, cardiovascular and inflammatory disorders (Kaczmarczyk, Miller, & Freund, 2012). This is the first study to investigate the fractions of fibers in *S. malaccense* fruit, which is rich in insoluble fibers, being the soluble ones concentrated in the pulp (Table 1). The amount of total fibers found in a previous study (Maisuthisakul, Pasuk, & Ritthiruangdej, 2008) was lower than that shown in this study, probably because of different methods used, conditions of harvest, or even sample treatment. For instance, it is known that freeze-drying method, used in the present study, increases the share of the soluble fraction of fibers (Milala et al., 2013).

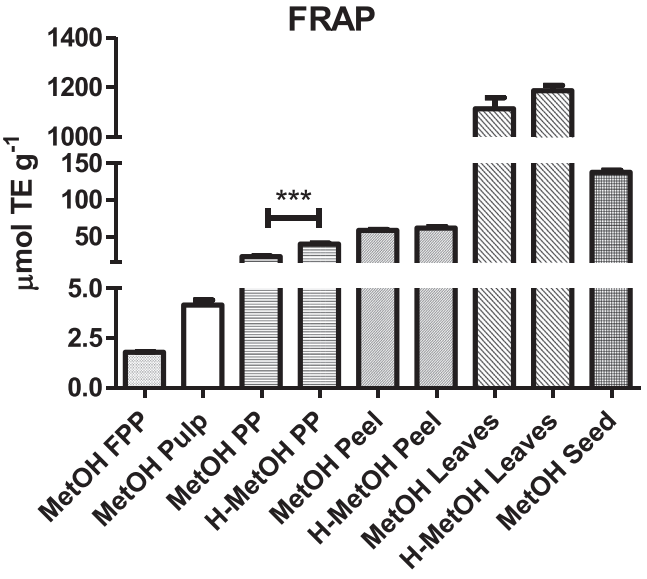

Fig. 1. Antioxidant capacity by FRAP assay in fruits and leaves of red-jambo. Samples were extracted with MetOH and acid hydrolysis (H-MetOH). PP: pulp + peel, FPP: fresh PP. Hydrolyzed extracts were compared to their respective MetOH using Student t-test (***$P < 0.001$).

The Folin-Ciocalteau method has shown a poor correlation with HPLC quantifications of phenolic compounds, since it could be affected by organic acids and reducing sugars (Kapasakalidis, Rastall, & Gordon, 2006). The red-jambo showed high acidity and amounts of reducing sugars and this might have influenced the result of total phenolic compounds by this method. The error could be minimized by using other techniques such as solid-phase extraction, although it could also reduce the final content of phenolic compounds (Palma, Pineiro, & Barroso, 2002). Particularly in this study, Folin-Ciocalteau reagent showed mild reaction with BHT, synthetic antioxidant used to reduce the heating damage when the extracts were hydrolyzed. Furthermore, previous works demonstrated no differences for Folin-Ciocalteau reagent reducing substances, ORAC (Batista et al., 2014) and FRAP assays (Batista et al., 2016) after acid hydrolysis of the MetOH fruit extracts containing 0.26 g L^{-1}BHT in the final solution. Based on this, we concluded that the use of BHT did not interfere on the statistical differences found for total phenolic compounds or antioxidant capacity values, since its reducing power was small.

Acid hydrolysis is a common technique used previously to phenolic compounds quantification by HPLC. The conditions used to hydrolyze extracts have been cited as: 1.2–1.5 mol L^{-1} HCl solution under 90 °C, during 60–120 min (Careri, Elviri, Mangia, & Musci, 2000; Kapasakalidis et al., 2006; Pyrzynska & Biesaga, 2009). These studies have shown release of phenolic monomers, anthocyanidins (aglycone forms of anthocyanins) and other flavonoid aglycone forms after hydrolysis. On the other hand, some deglycosylated phenolic compounds are labile molecules and may be degraded upon exposure to heat (Careri et al., 2000). Using 30 min, this study showed complete hydrolysis of some compounds, such as isorhamnetin-3-O-glucoside, isoquercitrin, (−)-epicatechin gallate and procyanidin B1; supported by the increased amounts of quercetin, (+)-catechin and (−)-epicatechin as aglycones/monomers of the last three compounds. Corroborating this data, the hydrolyzed peel extract showed high and positive correlation with these monomers as well as with anthocyanins, when PCA was used (Fig. 2).

The hydrolyzed extracts showed higher amounts of

Table 4
Polyphenols of *S. malaccense* fruit parts analyzed by HPLC-DAD/FLD expressed as mg 100 g^{-1} wet weight of freeze-dried samples.

Polyphenols		MetOH PP	H-MetOH PP	MetOH peel	H-MetOH peel
Phenolic acid					
1	*p*-Coumaric acid	ND	0.999 ± 0.000	ND	1.160 ± 0.001
2	Benzoic acid	0.724 ± 0.064	ND	1.517 ± 0.094	ND
Flavanols and Procyanidins					
3	(−)-Epicatechin gallate	1.374 ± 0.247	ND	1.21 ± 0.15	ND
4	(+)-Catechin	1.124 ± 0.087	0.500 ± 0.01***	0.224 ± 0.029	1.243 ± 0.096***
5	(−)-Epicatechin	1.374 ± 0.029	1.332 ± 0.083	0.808 ± 0.047	2.321 ± 0.167***
6	Procyanidin A2	1.736 ± 0.246	1.221 ± 0.192*	0.932 ± 0.025	0.622 ± 0.083***
7	Procyanidin B1	1.311 ± 0.246	ND	2.072 ± 0.189	ND
8	Procyanidin B2	0.450 ± 0.001	0.541 ± 0.083	1.206 ± 0.024	1.160 ± 0.192
Flavonols					
9	Isorhamnetin-3-*O*-glucoside	3.334 ± 0.205	ND	7.143 ± 0.141	ND
10	Isoquercitrin	0.549 ± 0.000	ND	2.014 ± 0.094	ND
11	Quercetin	0.200 ± 0.000	1.332 ± 0.136***	0.298 ± 0.000	3.646 ± 0.164***
12	Kaempferol-3-*O*-glucoside	1.315 ± 0.161	3.538 ± 0.250***	2.967 ± 0.077	1.878 ± 0.254**
13	Rutin	0.549 ± 0.100	1.082 ± 0.288*	0.448 ± 0.000	0.580 ± 0.096
Anthocyanins[a]					
14	Cyanidin-3,5-*O*-diglucoside[1]	5.519 ± 0.247	6.050 ± 0.535	11.698 ± 0.667	14.971 ± 0.671**
15	Cyanidin-3-*O*-glucoside[2]	42.77 ± 3.36	63.60 ± 0.99***	155.29 ± 3.76	226.54 ± 6.81***
16	Peonidin-3-*O*-glucoside[3]	0.512 ± 0.025	0.888 ± 0.096***	1.724 ± 0.124	3.066 ± 0.165***
	Sum	63.53 ± 5.10	81.02 ± 1.55**	189.53 ± 3.17	257.21 ± 6.14***

[a] Identifications of the anthocyanins:[1][M+] 611 *m/z*, MS/MS 449, 287 *m/z*, for cyanidin 3,5-*O*-diglucoside (TR = 1.6 min);[2][M+] 449 *m/z*, MS/MS 287 *m/z*, for cyanidin 3-*O*-glucoside (TR = 2.15 min) and[3][M+] 463 *m/z*, MS/MS 301 *m/z*, for peonidin and hexose. ND: non-detected. PP: pulp + peel. Hydrolyzed extracts were compared to their respective 80% MetOH extract using Student t-test (*$P < 0.05$,**$P < 0.01$, and***$P < 0.001$).

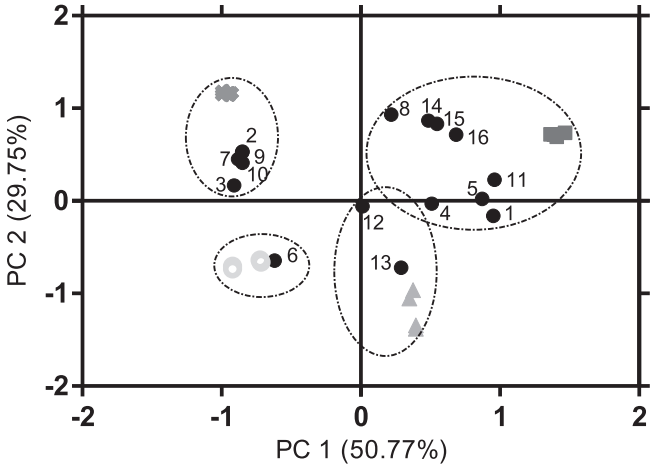

Fig. 2. Plot of the first and second PC score vectors of the 16 phenolic compounds found in the red-jambo fruit parts. The grouping indicates a relationship between the compounds marked as numbers: ●1: p-Coumaric, 2: benzoic acid, 3: (−)-epicatechin gallate, 4: (+)-catechin, 5: (−)-epicatechin, 6: procyanidin A2, 7: procyanidin B1, 8: procyanidin B2, 9: isorhamnetin-3-*O*-glucoside, 10: isoquercitrin, 11: quercetin, 12: kaempferol-3-*O*-glucoside, 13: rutin, 14: cyanidin-3,5-*O*-diglucoside, 15: cyanidin-3-*O*-glucoside, 16: peonidin-3-*O*-glucoside, and the method of extraction used for pulp + peel and peel, marked as ○ for MetOH PP, ▲H-MetOH PP, **x**MetOH Peel and ■H-MetOH Peel extracts.

anthocyanins, probably due to their stability in acid conditions. As demonstrated in another study (Lee, Rennaker, & Wrolstad, 2008), the pH differential method for monomeric anthocyanins quantification in this work showed strong correlation with HPLC results ($r = 0.997$, $P < 0.001$); however, the first method showed around 2.5 times higher values. Hence, even with more stability of anthocyanins in the H-MetOH extract, the quantification was possibly underestimated, since hydrolysis to anthocyanidin forms might have occurred concomitantly.

Even though, there are no previous works about the anthocyanins profile of red-jambo fruit by HPLC or MS. A study with Myrtaceae fruits (Reynertson et al., 2008) only showed the content of cyanidin 3-glucoside in the freeze-dried PP of *S. malaccense*, in lower amounts than that found in the present work. Furthermore, in agreement with our data (Table 4), quercetrin and rutin were also found in such study (Reynertson et al., 2008), but not ellagic acid.

There is no data about phenolic compounds in the separated parts of red-jambo and the present study has shown interesting results. For example, analyzing the peel and PP separately, it was possible to infer, that from the edible part, the pulp have the highest association with procyanidin A2 and kaempferol-3-*O*-glucoside and rutin, as shown by PCA (Table 4, Fig. 2).

Proanthocyanidins or condensed tannins were found in red-jambo, particularly in the non-hydrolyzed extract of the peel, as indicated by the PCA analysis (Fig 2). They represent oligomers or polymers of mainly (−)-epicatechin and (+)-catechin, which occur naturally in berries and represent the flavonoids more consumed everyday. They could be absorbed as native form or metabolized by gut microbiota, linked with prebiotic, antioxidant, antitumor and anti-inflammatory activities (Ou & Gu, 2014). In the PP of red-jambo, flavanols and procyanidins represent 11% of the phenolic compounds (Table 4).

The seed of red-jambo fruit is common wasted; however, it could be used for further investigations due to its antioxidant capacity and bioactive compounds, which were not previously studied. The interest in the use of byproducts, such as peels and seeds of fruits, is raising since they have shown higher antioxidant power and phenolic compounds content than in edible portions (Soong & Barlow, 2004). Our results of the seed analyses corroborated these findings.

The leaves of *S. malaccense* are known by their anti-inflammatory power (Andersson Dunstan et al., 1997) and this could be related to its elevated content of flavonoids, like myricitrin (Arumugam et al., 2014), and quercetin, found in the present study. Due to its several electron-donating groups, quercetin also pronounces antioxidant activity and could inhibit inflammation, likewise anthocyanins (Nair et al., 2006). Sources of quercetin, were associated with cardiovascular, antioxidant, and cognitive improvements (Williamson & Manach, 2005). The intake of edible red-jambo may well also be implicated with such effects, since quercetin was also found in the fruit.

Measurements of antioxidant capacity stimulate the consumption of plant-based foods, since they could predict 'in vivo' effects against oxidative stress (Paredes-López et al., 2010; Prior et al., 2003) (Batista et al., 2014). However, due to peculiar characteristics of each method (e.g. pH, temperature, and radical), they often show different results. In this work, among the methods used to determine antioxidant capacity, the ORAC assay (pH 7.4, 37 °C, peroxyl radical) was more sensible to the changes associated to the hydrolysis in comparison to the FRAP assay (pH 3.6, 37 °C, ferric reducing power). In addition, the use of DPPH assay (room temperature, DPPH radical) for berries may have controversial outcomes since the color of anthocyanins could influence the absorbance of the DPPH solution.

Finally, lipophilic compounds, such as carotenoids (Lako et al., 2007), and terpenes (Karioti, Skaltsa, & Gbolade, 2007; Pino et al., 2004) also contribute to the antioxidant effects of red-jambo fruit and leaves. However, the composition of carotenoids in red-jambo fruit remain unclear, since some studies either found β-carotene in the edible parts (Lako et al., 2007), either not (Khoo, Ismail, Mohd-Esa, & Idris, 2008). Nevertheless, the total carotenoids results indicate the importance of these compounds in the fruit (Khoo et al., 2008). The peel of red-jambo fruit might concentrate carotenoids and other lipids from the fruit (as suggested by total lipids, Table 1), which could explain the highest value found in the lipophilic antioxidant capacity.

This work showed that *S. malaccense* contains several bioactive compounds that could be used in the search of dietary therapies to help prevent certain diseases or maintain oxidative balance.

5. Conclusions

The fresh pulp and peel of red-jambo showed acid characteristics, elevated moisture content, and solid part containing bioactive compounds with nutritional and pharmacological interest. Each part of *S. malaccense* could be highlighted for different chemical characteristics: the pulp was a rich source of soluble fibers and reducing sugars; the peel concentrated the insoluble fibers, lipid content, lipophilic/hydrophilic antioxidant power, and anthocyanins; the seeds could be also highlighted by both lipophilic and hydrophilic antioxidant power; and finally the leaves that provide large amounts of catechins, quercetin, carotenoids, and great antioxidant capacity.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2016.05.013.

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