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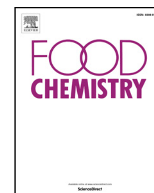
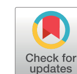


Chemical composition, nutritional properties, and antioxidant activity of *Licania tomentosa* (Benth.) fruit



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ABSTRACT

Licania tomentosa is a Brazilian plant species that produces edible fruits, yet there is little information available concerning their nutritional and/or bioactive composition. This study aimed to evaluate the nutritional and polyphenol composition of *L. tomentosa* fruits (pulp and seeds) and measure antioxidant activity in ethanolic extracts.The pulp and seeds were excellent sources of fiber (25.62%–41.70%) as well as minerals and vitamins. *L. tomentosa* contained no lectins or protease inhibitors (chymotrysin and trypsin) and 12 polyphenol compounds were identified in the seed extracts with a predominance of flavonoids. The seeds also presented antioxidant activities using the DPPH (SC_{50}10.30–15.87 μg/mL), TBARS (IC_{50} 18.46–20.84 μg/mL), and FRAP (RC_{50} 0.203–0.309 μg/mL) assays. Due to its nutrient and antioxidant content, *L. tomentosa* may be used for food applications.

1. Introduction

Brazil is a country of remarkable biodiversity. Its native vegetation is a rich source of bioactive molecules for food, pharmacology and other applications. Due to their rich supply of vitamins, minerals and bioactive molecules (e.g. phenolic compounds), regular consumption of fruits and vegetables presents many health benefits (Chang, Alasalvar, & Shahidi, 2016), yet many Brazilian plant species, including edible fruits, are still largely unknown and not fully characterized (Almeida et al., 2017).

Licania tomentosa (Benth.) is popularly known in northeastern Brazil as '*oiti*' or '*oitizeiro*'; the tree is a native species of the Atlantic Rain Forest (Agra, Freitas, & Barbosa-Filho, 2007). Its fruit is edible and presents as a fusiform or oval drupe (12–16 cm in length) with yellow peel (when ripe) and contains oil-rich seeds (Moreira-Araújo et al., 2019). The species is considered an unconventional food plant (UFP), although it is consumed fresh on a small scale or in the production of jams, jellies and flours (Kinupp & Lorenzi, 2014; Sousa, Silva, Sousa, Martins, & Gomes, 2013). However, although some studies have been carried out on the fruits to quantify nutritional and physico-chemical properties, and encourage consumption, nutritional studies concerning *L. tomentosa* pulp and seeds are scarce (Sousa et al., 2013). A previous study carried out by our group investigating the antibacterial, anticholinesterase and antioxidant activities of seeds from 21 Brazilian plants revealed *L. tomentosa* as a species with excellent antioxidant activity, due to its content of polyphenols and, more precisely, flavonoids (Farias et al., 2013; Pessoa et al., 2016).

The aim of this study was characterization of *L. tomentosa* fruits, with quantification and identification of phenolic compounds, and assessment of antioxidant capacity in ethanolic extracts of the pulp and seeds. This preliminary study may highlight the potential of this little-known plant species, while also promoting conservation of the Brazilian flora.

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2. Materials and methods

2.1. Chemicals

All chemicals used were of analytical grade. Argon was acquired from Air Liquide (São Paulo, Brazil). Ascorbic acid, quercetin, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropane (MDA), bovine serum albumin (BSA), fraction V free fatty acid, tryspin, chymotrypsin and protein substrate azocasein were acquired from Sigma-Aldrich (St. Louis, MO, USA). Na, K, P, Mg, Ca, Mn, Cu, Fe, Se and Zn were acquired from Merck (Darmstadt, Germany).

2.2. Plant material

Samples of *L. tomentosa* fresh fruit were collected at harvest in January 2015 and 2016 from *Campus* do Pici, Fortaleza, Ceará State (3°44′32.6″S 38°34′30.5″W). A total of 50 fruits, taken from three trees, were collected each year. The average weight of the fruits was 91.54 ± 23.87 (2015) and 85.43 ± 18.69 g (2016) with lengths of 7.36 ± 0.92 and 7.11 ± 0.81 cm for 2015 and 2016, respectively.

The *L. tomentosa* fruit is a drupe, composed of a thin, soft skin (exocarp) covering a soft, oily pulp (mesocarp), surrounding a thin pit (endocarp) with a large seed inside, which accounts for most of the fruit's weight.

After collection, the *L. tomentosa* branches with inflorescences and fruits were dehydrated and deposited at the Prisco Bezerra Herbarium (access number EAC 40215), located at the Federal University of Ceara - UFC (Fortaleza, Brazil).

The seeds were separated from the skin and pulp, and both were oven dried (FANEM, Model 002 CB, São Paulo, Brazil) at 50 °C for 24 h. Then, the seeds and pulp were ground in a coffee grinder (Cadence, Itajaí, Brazil) to obtain a fine powder (particle size < 0.5 mm), which was stored at room temperature (25 °C) in hermetically sealed plastic pots until analysis. For nutritional composition, the pulp and seed flours collected in 2015 and 2016 were labeled as follows: LTP1, *L. tomentosa* pulp flour collection 1 (2015); LTP2, *L. tomentosa* pulp flour collection 2 (2016); LTS1, *L. tomentosa* seed flour collection 1 (2015); and LTS2, *L. tomentosa* seed flour collection 2 (2016).

2.3. Nutritional assessment

2.3.1. Proximate composition

The pulp and seeds were analyzed for moisture, ash and total lipid contents in accordance with Association of Analytical Communities (Association of Official Analytical Chemists (AOAC), 2012) methods. After acid digestion (Marconi equipment, MA 448, Piracicaba, Brasil), followed by photocolorimetric assay (Baethgen & Alley, 1989), nitrogen was estimated using the micro-Kjeldahl method (AOAC, 2012). Total protein content was calculated using the conversion factor $N \times 6.25$. Total dietary fiber was determined using an enzymatic kit (TDF-100A, Sigma–Aldrich Co., MO, USA) following the Prosky-AOAC method (AOAC, 2012). Digestible carbohydrate content was calculated from the percentage difference ratio to all other constituents, according to the formula:

$$[100 - (\%\ crudeprotein\ +\ \%\ crude\ lipid\ +\ \%\ ash\ +\ \%\ dietary\ fiber)].$$

2.3.2. Toxic and anti-nutritional factors

For determination of lectins, trypsin, and chymotrypsin inhibitors, crude aqueous extracts of the pulp and seeds were prepared according to Vasconcelos et al. (2010).

The presence of lectins in the crude extracts was assayed based on hemagglutinating activity using 1% erythrocyte suspensions of rabbit and rat bloods, and treated or not with trypsin (10 mg L^{-1}), as described by Vasconcelos et al. (2010). The results are reported as hemagglutination titer (HU), which is the reciprocal of the highest dilution yielding visible agglutination. The assay was approved by the Ethics Commission on Animal Research (CEUA) of the Federal University of Ceara (Fortaleza, Brazil).

Inhibitory activity against chymotrypsin and trypsin was determined using an azocasein substrate (Erlanger, Kolowsky, & Cohen, 1961). The activities are expressed as inhibition units × mg of protein^{-1}, which was monitored by absorbance (0.01) at 440 nm. The toxic and anti-nutritional factor detection assays were performed in triplicate.

2.3.3. Mineral composition

2.3.3.1. Microwave assisted closed digestion system. The mineral composition was determined according to AOAC (2012). A closed microwave digestion system (Start D, Milestone, Sorisole, Italy) was used to determine the minerals as follows: 500 mg of sample was weighed into a PTFE digestion flask, and 8 mL concentrated HNO_3 and 2 mL H_2O_2 were added. The flasks were sealed and transferred to a microwave digester. Four heating gradients were used to apply 1,000 W: (a) from room temperature to 70 °C in 5 min; (b) from 70 to 120 °C in 5 min; (c) from 120 to 170 °C in 5 min; and (d) maintaining 170 °C for 25 min. After cooling, the flasks were opened, and the resulting solution transferred to a 25 mL Falcon tube in 5% (v/v) HNO_3.

2.3.3.2. Determining Ca, Se, Cu, Fe, P, Mg, Mn, K, Na, Zn using (ICP-OES). Minerals were determined using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES), model 5100 VDV (Agilent Technology, Tokyo, Japan) equipped with a double pass nebulization camera and a spray nebulizer. Liquid argon was used, as both the nebulizing gas and auxiliary gas, to generate plasma to determine Ca, Se, Cu, Fe, P, Mg, Mn, K, Na and Zn. The optimized conditions were as follows: radiofrequency generator power (1,200 W); argon flow rate (12 L/min); auxiliary argon flow rate (1 L/min Air); seaspray concentric nebulizer with flow rate of 0.50 L/min; axial view of plasma; number of replicates n = 3/sample; and wavelengths: Ca 317.933 nm; Se 196.026 nm; Cu 324.754 nm; Fe 259.940 nm; K 766.491 nm; Mg 279.553 nm; Mn 257.610 nm; Na 589.592 nm; P 213.618 nm and Zn 206.200 nm.

The analytical curves were prepared from standard certified solutions at: 10,000 mg L^{-1} for Na, K, P, Mg, Ca, and 1000 mg L^{-1} for Mn, Cu, Fe, Se and Zn (Merck, Darmstadt, Germany) in 5% (v/v) HCl solution. The minerals were determined by ICP-OES, using the external calibration method, with analytical curves prepared from solutions at the following concentrations: 0.04 to 41 mg 100 g^{-1} for Na and Ca; 0.06 to 61 mg 100 g^{-1} for P and K; 0.001 to 1.000 for Fe, Cu, Se, Mn and Zn; 0.00025 to 0.1000 mg 100 g^{-1} for Se, and 0.015 to 14.5 mg 100 g^{-1} for Mg.

2.3.4. Vitamin E and carotenoids

Tocopherols and vitamin E were determined according to Brubacher, Mueller-Mulot, and Southgate (1985). Briefly, about 2 g of each sample (LTP1, LTP2, LTS1, and LTS2) were saponified in 50% hot potassium hydroxide, at a temperature between 80 and 90 °C, for 30 min. Unsaponifiable material was extracted sequentially from the matrix with a ethyl ether: petroleum ether: ethyl acetate (63:32:05, v/v/v) mixture. The fractions were combined and washed with deionized water until neutral pH was achieved. The extract was dried under nitrogen flow and diluted in *n*-hexane. Detection and quantification of tocopherols was performed in a Prominence LC-20A Liquid Chromatograph coupled to a fluorescence detector; RF-10AXL (Shimadzu, Tokyo, Japan), using an excitation wavelength of 292 nm and emission wavelength of 336 nm. The analytes were resolved on a LiChrospher Si 60 normal phase column (12.5 cm length × 4 mm diameter, and 5 µm particles, Merck, Germany) with the mobile phase *n*-hexane: ethyl acetate: acetic acid (97.6: 1.8: 0.6, v/v/ v) in an isocratic system.

Carotenoids were determined according to Carvalho, Collins, and

Rodriguez-Amaya (1992). Intriplicate, the samples 2 g were hydrated and pigments extracted with portions of acetone (30 mL) in a homogenizer (Marconi, TE 102, Piracicaba, Brazil) until the acetone turned colorless. The pigments were transferred to petroleum ether and the acetone removed by addition of water. The extract was concentrated and the volume adjusted to 10 mL with petroleum ether. Carotenoid quantification was performed at 453 nm in a Cary 50 Spectrophotometer (Varian, Victoria, Australia), using a value of 2592 absorbance units. All assays were performed in triplicate.

2.4. Chemical characterization of the pulp and seed ethanolic extracts

2.4.1. Ethanolic extract preparation

The pulp and seeds were separately oven dried at 50 °C; then ground and immersed separately (prolonged extraction) in ethanol PA (1:2 m/v), at room temperature with solvent changes every three days, following the methodology described by Farias et al. (2013), with modifications. The extracts were passed through Whatman® No.1 filter paper to remove minor impurities, and the solvent evaporated in a rotary evaporator under reduced pressure (20 cmHg) and controlled temperature (50 °C), with subsequent removal of excess ethanol in a water bath. The ethanolic extracts obtained were stored in tubes protected from light at −20 °C until analysis. The ethanolic pulp and seed extracts, collected in 2015 and 2016, were logged as follows: EELTP1, ethanolic extract from the *L. tomentosa* pulp collection 1 (2015); EELTP2, ethanolic extract from the *L. tomentosa* pulp collection 2 (2016); EELTS1, ethanolic extract from the *L. tomentosa* seed collection 1 (2015); and EELTS2, ethanolic extract from the *L. tomentosa* seed collection 2 (2016).

2.4.2. Total phenols and flavonoids

Total phenol content was determined following the Folin-Ciocalteu method as described by Pessoa et al. (2016). For flavonoids, the aluminum chloride colorimetric method was used, as described by Chang, Yang, Wen, and Chern (2002). Assays were performed in triplicate, and the results for total phenols and total flavonoids were expressed as mg of quecertin/mg of sample and as mg of gallic acid equivalent/g of sample, respectively.

2.4.3. Identification of phytochemicals using ultra performance liquid chromatography (UPLC)

Identification of the phytochemicals in EELTP1, EELTP2, EELTS1, EELTS2 was performed using an UPLC system (Waters) coupled to a QuadripoleSystem/Time of Flight (QtoF, Waters). Chromatographic runs were performed in a Waters Acquity UPLC BEH column (150 × 2.1 mm, 1.7 um), with temperature fixed at 40 °C, mobile water phases with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), a gradient ranging from 2 to 95% B (15 min), a flow rate of 0.4 mL/min, and an injection volume of 5 µL.

The ESI − mode was acquired using a range of 110–1180 Da; source temperature set at 120 °C, desolvation temperature 350 °C, desolvatation gas flow of 500 L/h, extraction cone at 0.5 V, and capillary voltage at 2.6 kV. ESI + mode was acquired using a range of 110–1180 Da, a fixed source temperature at 120 °C, a desolvation temperature of 350 °C, desolvatation gas flow of 500 L/h, and capillary voltage of 3.2 kV. Leucine enkephalin was used as a lock mass. The acquisition mode was MS^E. The instrument was controlled by Masslynx 4.1 software (Waters Corporation, Waters, Milford, USA)

2.4.4. In vitro antioxidant activity

2.4.4.1. DPPH radical scavenging. The antioxidant activity of the seed and pulp ethanolic extracts was evaluated using the photo-colorimetric DPPH method, based on scavenging capacity to neutralize DPPH free radicals (Zhang et al., 2014). SC_{50} (the sample concentration must scavenge 50% of available DPPH radical) was obtained by plotting DPPH-scavenging percentage for each sample against sample concentrations. Ascorbic acid was used as the positive control.

2.4.4.2. TBARS assay. TBARS production was determined using egg yolk phospholipids according to the method described by Sabir and Rocha (2008). TBARS are expressed in µg MDA/mLof sample.

2.4.4.3. FRAP assay. The FRAP assay was performed as described by Benzie and Strain (1999). A calibration curve was constructed using different concentrations of ferrous sulfate heptahydrate (0–2,000 mM), and the results are expressed in mM of ferrous sulfate/mg of seed and pulp ethanolic extract. All assays for antioxidant capacity were performed in triplicate for each sample.

2.5. Statistical analysis

Results are expressed as mean ± standard deviation. To evaluate degrees of similarity between variables, simple comparisons tests (*t*-test) were performed. Differences were considered statistically significant at $p < 0.05$. For certain assays, the results are expressed as EC_{50} (median effective concentration), which represents concentration at which half (50%) of themaximal effect was observed and expressed as SC_{50} ("S" for Scavenging), and IC_{50} ("I" for inhibition).

3. Results and discussion

The proximate composition of seeds and pulp is shown in Table 1. We observed significant differences in contents between samples collected in 2015 and 2016. It is well established that the nutritional value and bioactive potential of plants are determined by production and accumulation of primary and secondary metabolites, which may differ among plants of the same species due to differing environmental conditions (Sampaio, Edrada-Ebel, & Costa, 2016). The only condition that

Table 1
Moisture and proximate composition on a dry basis (%) of *Licania tomentosa* pulp and seed flours.

	LTS1	LTS2	LTP1	LTP2
Moisture	37.39 ± 0.37[a]	38.18 ± 1.19[a]	73.82 ± 1.29[a]	76.02 ± 0.67[a]
Protein[1]	3.10 ± 0.10[a]	3.47 ± 0.13[b]	3.82 ± 0.03[a]	4.59 ± 0.16[b]
Fat	0.59 ± 0.01 [a]	0.90 ± 0.02[b]	3.34 ± 0.08[a]	4.08 ± 0.08[b]
Dietary Fiber	25.62 ± 0.17[a]	27.45 ± 0.2[b]	39.18 ± 0.28[a]	41.70 ± 0.24[b]
Ash	1.68 ± 0.02[a]	1.76 ± 0.04[b]	2.75 ± 0.04[a]	2.86 ± 0.03[b]
Digestible Carbohydrate[2]	69.01[a]	66.42[b]	50.91[a]	46.77[b]

Mean ± SD values of fruit and seed followed by different letters in the same row; significant differences ($p < 0.05$).
LTS1: *L. tomentosa* seed flour collection 1 (2015); LTS2: *L. tomentosa* seed flour collection 2 (2016); LTP1: *L. tomentosa* pulp flour collection 1 (2015); LTP2: *L. tomentosa* pulp flour collection 2 (2016).
[1] N × 6.25;
[2] The available digestible carbohydrate content was determined by calculating the percentile difference from all the other constituents according to the formula: [100 g dry weight - (g crude protein + g crude lipid + g ash + g dietary fiber)]/100 g;

Table 2
Vitamins in *Licania tomentosa* pulp and seed flours.

	LTS1	LTS2	LTP1	LTP2
Tocopherol (mg/100 g)				
Alpha (α)	0.74 ± 0.0[a]	0.38 ± 0.02[b]	0.02 ± 0.00[a]	0.06 ± 0.00[b]
Beta (β)	ND < 0.01	ND < 0.01	ND < 0.01	ND < 0.01
Gamma (γ)	1.02 ± 0.02[a]	0.33 ± 0.00[b]	0.04 ± 0.00[a]	0.14 ± 0.00[b]
Delta (Δ)	ND < 0.01	ND < 0.01	ND < 0.01	ND < 0.01
Total	1.76 ± 0.0[a]	0.71 ± 0.02[b]	0.06 ± 0.00[a]	0.20 ± 0.01[b]
Vitamin E				
UI/100 g	1	< 1	< 1	< 1
Expressed as α-tocopherol (mg/100 g)	0.88 ± 0.02[a]	0.43 ± 0.01[b]	0.02 ± 0.00[a]	0.08 ± 0.00[b]
Total carotenoids (mg/100 g)				
Expressed such as beta-carotene	ND	ND	ND	ND

Mean ± SD values of fruit and seed followed by different letters in the same row significant differences ($p < 0.05$). ND: not detected
LTS1: *L. tomentosa* seed flour collection 1 (2015); LTS2: *L. tomentosa* seed flour collection 2 (2016); LTP1: *L. tomentosa* pulp flour collection 1(2015); LTP2: *L. tomentosa* pulp flour collection 2(2016).

Table 3
Minerals found in *Licania tomentosa* pulp and seed flours.

Elements (mg/100 g)	LTS1	LTS2	LTP1	LTP2
Calcium	71.47 ± 0.12[a]	86.76 ± 0.59[b]	167.8 ± 1.69[a]	189.7 ± 1.23[b]
Selenium	0.04 ± 0.00[a]	0.01 ± 0.00[b]	0.06 ± 0.00[a]	0.04 ± 0.00[b]
Copper	0.73 ± 0.00[a]	0.49 ± 0.00[b]	0.77 ± 0.00[a]	0.66 ± 0.00[b]
Iron	0.77 ± 0.02[a]	1.40 ± 0.24[b]	1.09 ± 0.01[a]	1.20 ± 0.00[b]
Phosphorous	50.60 ± 0.06[a]	58.63 ± 0.48[b]	54.87 ± 0.45[a]	60.50 ± 0.40[b]
Magnesium	61.04 ± 0.24[a]	70.43 ± 0.32[b]	83.35 ± 0.89[a]	79.19 ± 0.50[b]
Manganese	0.29 ± 0.00[a]	0.31 ± 0.00[b]	0.49 ± 0.00[a]	0.34 ± 0.00[b]
Potassium	588.90 ± 1.01[a]	643.1 ± 6.88[b]	887.3 ± 8.35[a]	943.9 ± 7.28[b]
Sodium	22.61 ± 0.10[a]	16.26 ± 0.21[b]	144.8 ± 1.23[a]	111.1 ± 0.78[b]
Zinc	0.56 ± 0.01[a]	0.49 ± 0.00[b]	0.56 ± 0.00[a]	0.56 ± 0.02[a]

Mean ± SD values for fruit and seed followed by different letters in the same row; significant differences ($p < 0.05$).
LTS1: *L. tomentosa* seed flour collection 1 (2015); LTS2: *L. tomentosa* seed flour collection 2 (2016); LTP1: *L. tomentosa* pulp flour collection 1(2015); LTP2: *L. tomentosa* pulp flour collection 2 (2016).

Table 4
Total polyphenol and flavonoid content of *Licania tomentosa* pulp and seed ethanolic extracts.

Phenolic compounds	EELTS1	EELTS2	EELTP1	EELTP2
Total Polyphenols[1]	192.40 ± 3.12[a]	203.00 ± 1.44[a]	ND	ND
Flavonoids[2]	29.29 ± 0.36[a]	29.88 ± 0.48[a]	19.80 ± 1.57[a]	20.67 ± 0.17[a]

Mean ± SD values of fruit and seed followed by different letters in the same row; significant differences ($p < 0.05$).
ND: not detected.
EELTP1: ethanolic extract from *L. tomentosa* pulp collection 1 (2015); EELTP2: ethanolic extract from *L. tomentosa* pulp collection 2 (2016); EELTS1: ethanolic extract from *L. tomentosa* seed collection 1 (2015); EELTS2: ethanolic extract from the *L. tomentosa* seed collection 2 (2016).
[1] Gallic acid equivalent in mg per mg of sample.
[2] Quercetin equivalent in mg per mg of sample.

might have contributed to the differences observed in nutrient contents was the rainfall pattern. This is supported by official precipitation data, where a higher rainfall index was recorded in 2016 compared with 2015, and a difference in rainfall distribution during the rainy season from January through May (FUNCEME, 2018).

Both pulp (LTP1 and LTP2) and seed (LTS1 and LTS2) extracts presented values for lipids, proteins and ash similar (< 5%) to those observed previously (Ekpete, Edori, & Fubara, 2013). However, they remained excellent sources of dietary fiber (25% for seeds, and 41% for pulp), regardless of nutrient contents. Generally, fruits are excellent sources of dietary fiber and it is worth mentioning that '*oiti*' pulp presents values similar to or greater than many fruits consumed by the population worldwide such as plum (7.1%) and peach (8.2%) (Al-Farsi & Lee, 2008).

As for proteins (1.56%) and lipids (1.31%), proximate pulp composition results were similar to values described for apple pulp (Onivogui, Zhang, Mlyuka, Diaby, & Song, 2014). Ash values were lower (about 3%) than those for apple pulp (5.72%), but higher than averages for fruits in general (Ekpete at al., 2013). In relation to dietary fiber, a grape pulp study presented lower values (27.34%) than those observed in this study (about 40%) (Bampi, Bicudo, Oliveira, Fontoura, & Ribani, 2010). Proximate seed composition values were similar to those described for apples, with carbohydrates predominanting (71.11%), and less protein (2.7%) and ash (2.62%), but the lipid content was much lower than apple seeds (15.86%) (Onivogui et al., 2014).

Values for α- and γ-tocopherolin the seeds, (Table 2) in LTS1 (0.74 and 1.02 mg/100 g) and LTS2 (0.38 and 0.33 mg/100 g) were higher than those in the pulps, LTP1 (0.02 and 0.04 mg/100 g), and LTP2 (0.06 and 0.14 mg/100 g). The higher values for α-tocopherol in the seeds are similar to or higher than values for the pulps of many fruits. Chun, Lee, Ye, Exler, and Eitenmiller (2006) published values ranging from 0.02 mg/100 g in pineapple to 1.31 mg/100 g in kiwi. To the knowledge of the authors, no results for γ-tocopherol in fruits are available in the literature. β-, δ-tocopherol and the carotenoids were undetectable in both the pulp and seeds.

The vitamin E concentrations in the pulp (0.43 and 0.88 mg/100 g)

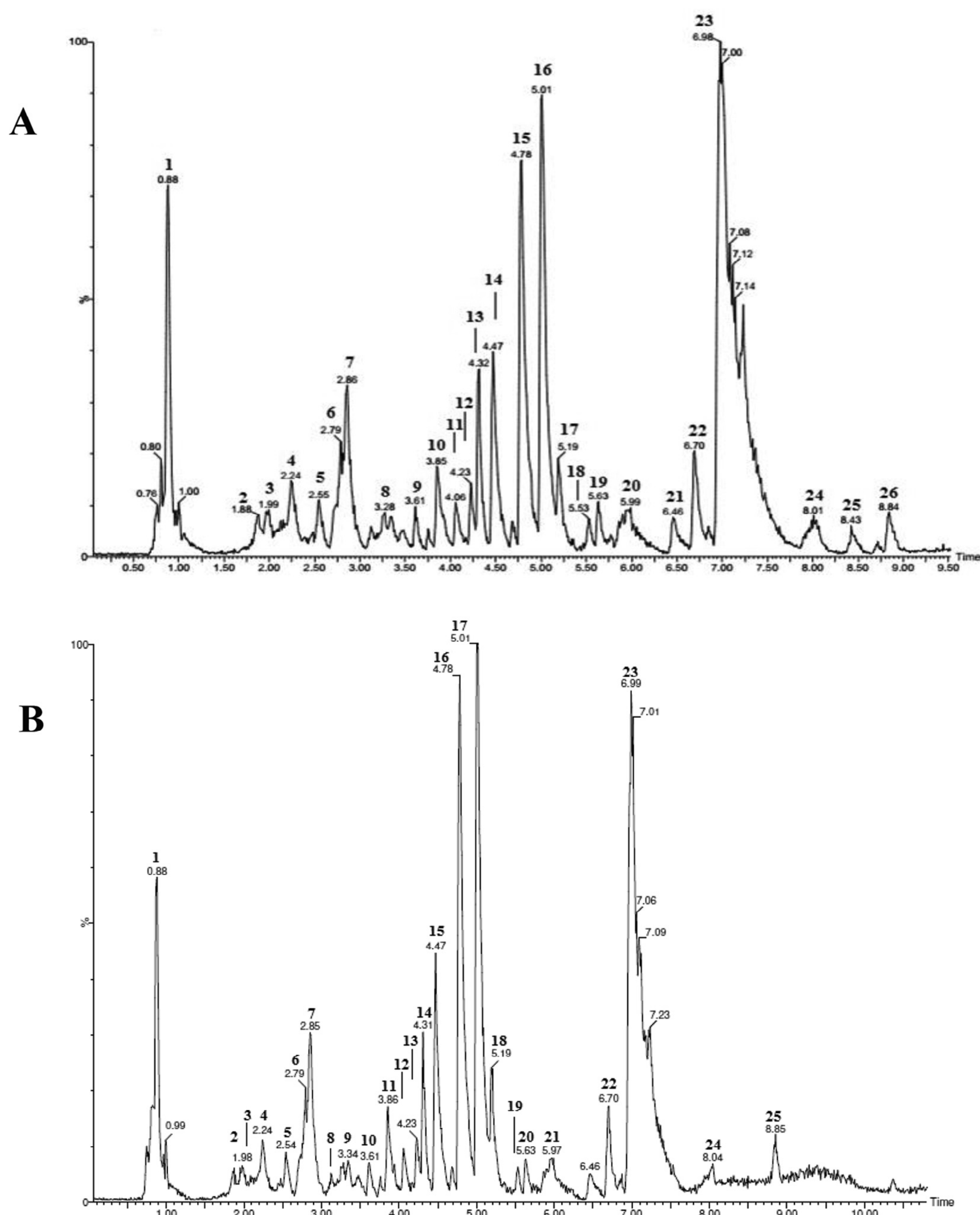

Fig. 1. UPLC chromatograms of *Licania tomentosa* seed ethanolic extract; harvest 1 (EELTS1) (A), and 2 (EELTS2) (B). A: 1 = Naphthalenedicarboxylic acid hexoside, 2 = Gallocatechin dimer isomer, 3 = Gallocatechin dimer isomer, 4 = Gallocatechin, 5 = Coumaric acid, 6 = Ferulic acid, 7 = Unknown, 8 = Hydroxy jasmonic acid-*O*-hexoside, 9 = Hydroxy-dihydrojasmonic acid-*O*-hexoside, 10 = Unknown, 11 = Lariciresinol hexoside, 12 = Quercetin pentosyl hexoside, 13 = Tinospinoside D isomer, 14–26 = Unknown. B: 1 = Naphthalenedicarboxylic acid hexoside, 2 = Gallocatechin dimer isomer, 3 = Gallocatechin dimer isomer, 4 = Gallocatechin, 5 = Coumaric acid, 6 = Ferulic acid, 7 = Unknown, 8 = Hydroxyjasmonic acid-*O*-hexoside isomer, 9 = Hydroxyjasmonic acid-*O*-glucoside isomer, 10 = Hydroxy dihydrojasmonic acid-*O*-hexoside, 11 = Unknown, 12 = Lariciresinol hexoside, 13 = Quercetin pentosyl-hexoside, 14 = Tinospinoside D isomer, 15–25 = Unknow.

Table 5
Antioxidant activity of *Licania tomentosa* seed ethanolic extract.

	DPPH assay SC_{50} (μg/mL)	FRAP (mM $FeSO_4$/mg extract)	TBARS assay IC_{50} (μg/mL) Without iron stress	With iron stress
EELTS1	15.87 ± 0.88^{c}	0.203 ± 0.00^{a}	18.46 ± 2.24^{a}	93.94 ± 2.49^{a}
EELTS2	10.30 ± 0.15^{b}	0.309 ± 0.01^{b}	20.84 ± 2.05^{a}	88.00 ± 2.08^{a}
Ascorbic Acid[1]	7.30 ± 0.05^{a}	–	–	

Values are means ± SD of three measurements. Values with different letters in the same column differ by Tukey test at $p < 0.05$. [1] Used as positive control. EELTP1: ethanolic extract from *L. tomentosa* pulp collection 1 (2015); EELTP2: ethanolic extract from *L. tomentosa* pulp collection 2 (2016); EELTS1: ethanolic extract from *L. tomentosa* seed collection 1 (2015); EELTS2: ethanolic extract from the *L. tomentosa* seed collection 2 (2016).

and seeds (0.02 and 0.08 mg/100 g) were low when compared to fruits recognized as good sources of α-tocopherol such as tomato fruit (6.0 mg/100 g) (Raiola, Tenore, Barone, Frusciante & Rigano, 2015). Nevertheless, due to the sheer abundance of plant-derived foods in our diets, a significant source of vitamin E is constantly provided. The richest dietary sources of vitamin E remain edible vegetable oils, which contain all of the different homologues in varying proportions (Rizvi et al., 2014). The total carotenoids determination for pulp and seeds also showed low values (below to the limit of detection) when compared, for example, to tomato fresh fruits (0.41 mg/100 g) (Perveen et al., 2015).

There was statistical difference between minerals determined in the pulps (LTP1 and LTP2) and the seeds (LTS1 and LTS2) from *L. tomentosa* (Table 3). In general, the pulps contained higher concentrations of minerals compared to the seeds, especially calcium, magnesium, potassium and sodium. Studying various fruits, Pires et al. (2015) recorded potassium, phosphorus, calcium, manganese, sodium, magnesium, iron, zinc, copper and selenium in mango pulp, Barbados cherries and strawberries, at values lower than those recorded here for *L. tomentosa* pulp. Thus, *L. tomentosa* is a good source of minerals, since values were similar to or higher than those of fruits consumed around the world.

As to the presence of toxic and/or anti-nutritional factors, no hemagglutinating activity was detected when tested with different types of blood (rabbit and rat), whether treated or not with trypsin, which would indicate the presence of lectins. An absence of lectins is positive since they normally do not degrade in the gastrointestinal tract and may interfere in nutrient absorption (Vasconcelos et al., 2010). The samples also had no inhibitory activity against trypsin and chymotrypsin. This is important because protease inhibitors complex with digestive enzymes, inhibiting their catalytic activity and impairing digestion. These result are positive when compared to gingerbread plum seeds (also belonging to Chrysobalanaceae), which was studied by Diaby, Amza, Onivogui, Zou, and Jin (2016), and found to contain trypsin inhibitors.

In addition to nutritional characterization, we considered phytochemical compounds and their relations hips with apparent antioxidant activities, subject to the method selected for analysis. Ethanolic extracts were prepared initially and yields were calculated [EELTS1 (26.56%), EELTS2 (24.90%), EELTP1 (15.87%), and EELTP2 (16.74%)]. Results are shown in Table 4 for phytochemical compounds identified using quantitative testing for total polyphenols and flavonoids. Determination of total polyphenols revealed similar phenolic concentrations in EELTS2 (203.00 ± 1.44 μg/mg) and EELTS1 (192.40 ± 3.12 μg/mg); polyphenols (total) were not detected in either EELTP1 or EELTP2. However, total flavonoids were detected in both EELTS1 and EELTS2, and were similar (at about 30 μg/mg) but higher than those determined in EELTP1 and EELTP2 (at about 20 μg/mg).

To elaborate the phytochemical profiles further, ethanolic pulp and seed extracts underwent UPLC-QTOF. The results (Fig. 1) showed similar profiles for seed samples with a total of 26 peaks, of which 11 were identified in EELTS1 and 12 in EELTS2. The various compounds identified in EELTS1 and EELTS2 (Supplementary Tables 1 and 2) were flavonoids (gallocatechin dimer isomer, gallocatechin and quercetin pentosyl-hexoside), phenolic acids (coumaric acid and ferulic acid), glycoside (hydroxy jasmonic acid-O-hexoside and hydroxy-dihydroxy-glucoside), lignan (lariciresinol hexoside), diterpene (tinospinoside D isomer), and organic acid (naphthalenedicarboxylic acid-hexose). Few studies have identified phytochemical compounds in *L. tomentosa*. Of the 11 phytochemical compounds identified, only catechin, quercetin and derivatives have been reported previously (Feitosa, Xavier, & Randau, 2012). UPLC-QTOF analyses of *L. tomentosa* pulp ethanolic extracts revealed 15 peaks, but none was identified.

Initial testing of EELTP1 and EELTP2 revealed no antioxidant activities above 1000 μg/mL using DPPH. Thus, we did not continue with this analysis. In contrast, ethanolic extracts of seeds presented promising results, as determined by the different methods (Table 5). SC_{50} values in the DPPH assay for EELTS1 and EELTS2 (15.87 ± 0.88 and 10.30 ± 0.15 μg/mL, respectively) were close to the positive control (i.e., ascorbic acid, 7.30 ± 0.05 μg/mL). Values were lower than those found for "Araçá" (*Psidium cattleianum*), and Purple pitanga (*Eugenia uniflora*) fruits, which were 48.05 ± 12.1, and 36.78 ± 5.8 μg/mL, respectively (Denardin et al., 2015).

To observe the capacity of samples to reduce ferric to ferrous ions, we used the FRAP method. This assay is important since iron (II) can participate in Fenton reactions generating hydroxyl radicals that are extremely reactive and can cause extensive molecular damage (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006). In the present study, EELTS2 (0.309 ± 0.01 mM) presented better results than EELTS1 (0.203 ± 0.00 mM), but values for both extracts were lower than those found for Japanese persimmon (*Diospyros kaki*) at 1.25 ± 0.04 mM (Celep, Aydin &Yesilada, 2012).

When considering the capacity for lipid peroxidation inhibition, using TBARS (with and without iron a stress inducing agent), no differences were observed among the samples. In the absence of iron, EELTS1 (18.46 ± 2.24 μg/mL) and EELTS2 (20.84 ± 2.05 μg/mL) presented higher results than those found by Pessoa et al. (2016) in *L. tomentosa* seed ethanolic extracts (236.07 ± 23.76 μg/mL). EELTS1 (93.94 ± 2.49 μg/mL) and EELTS2 (88.00 ± 2.08 μg/mL) had values similar to those found for *Malpighia glabra* fruits (112.54 ± 5.61 μg/mL) (Nascimento et al., 2018). Generally, flavonoids are associated with DPPH, FRAP and TBARS assay results (Dueñas, González-Manzano, González-Paramás, & Santos-Buelga, 2010). In this work, we have identified flavonoids such as quercetin and gallocatechin which may be responsible for the above mentioned results. Supporting this hypothesis, Pessoa et al. (2016) found for the *L. tomentosa* seed extract a high correlation coeficients between quercetin and DPPH activity (0.788), quercitrin and DPPH (0.745), and catechin and TBARS activity (0.690).

4. Conclusions

L. tomentosa pulp and seeds are demonstrated as good sources of dietary fiber and the mineral and vitamin contents are similar to or higher than those of fruits consumed more traditionally. In addition, *L. tomentosa* pulp and seeds do not contain anti-nutritional factors, such as lectins, and ethanolic seed extracts presented promising phenolic profiles (predominantly flavonoids) including compounds with high antioxidant activities. Thus, *L. tomentosa* fruit may be exploited for nutrient and antioxidant contents in food applications, which might offer local populations economic benefits provided biodiversity is also protected.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.126117.

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