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Chemical characterization of different parts of noni (*Morinda citrifolia*) fruit and its freeze-dried pulp powder with emphasis on its bioactive compounds and antioxidant activities

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Abstract

The aim of this study was to prepare the freeze dried powder of noni (*Morinda citrifolia*) pulp and determine the contents of bioactive and antioxidant capacity. Seed, peel, pulp and lyophilized powder were characterized in terms of physico-chemical, carotenoids, chlorophyll, sugars, ascorbic acid, phenolics, flavonoids and antioxidants contents. Phenolic compounds were determined by LC-MS. The lyophilized pulp powder showed high levels of phenolics and flavonoids (7486.38 μg GAE/g and 385.57 μg QE/g, respectively) and higher antioxidant activity for FRAP (10360.39 to 467970.40 mmol/100 g). Ascorbic acid has a higher concentration in the lyophilized powder (336.62 mg/100 g). Rutin, caffeic, artepillin C, quercetin-3-glucoside, kaempferol, vanillin and vanillic were the major compounds. The antioxidant effect present in the various components of the fruit was predominant, and the greater conservation of this potential was through the application of lyophilization. Thus, the noni may be considered as a good source of phenolics compounds having a high potential as natural antioxidants.

Keywords: *Morinda citrifolia*; bioactive compounds; antioxidants; freeze-drying.

Practical Application: In this study, noni parts were characterized and the results revealed that noni can be considered a good source of phenolic compounds with high potential as natural antioxidants.

1 Introduction

Noni (*Morinda citrifolia* L.) is native to Polynesia and is grown in tropical and subtropical countries as a sustainable crop (Jahurul et al., 2021). Noni seed is rich in phenols, flavonoids, carotenoids, vitamin C, tannin, polyunsaturated fatty acids, phytosterols, and tocopherols (Jahurul et al., 2021).

Noni is used by the pharmaceutical and cosmetic industries due to the pharmacological activities of its metabolites (Ruhomally et al., 2015) and has antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory and immunological properties (Abou Assi et al., 2017). In addition, Osorio et al. (2021) suggest that the chemical constituents present in the essential oil of *Morinda citrifolia* at 2% are of interest for its usage in the form of biofilms, prolonging the shelf life of perishable fruits stored at room temperature.

Fruits have been considered to be beneficial for health due to their high amounts of phenolic compounds, as they alleviate oxidative stress produced by free radicals and subsequently cell damage. These properties have increased the interest to investigate the antioxidant composition and function of fruits (Spínola et al., 2015). According to Narasingam et al. (2017), the noni fruit has anxiolytic and antidepressant activities; however, the exact bioactive principles responsible for these effects have not yet been elucidated. Studies on the bioactive compounds present in noni are deficient and need to be further explored, in order to verify which of the components of the fruit presents a more promising source of research to validate and demonstrate its biological potential which are related to problems of public health. The bioactive compounds in fruits can contribute to the benefits of the human body when administered regularly in the diet. In addition, these compounds are also related to the reduction of microbial and chemical deterioration (Prestes et al., 2022).

There are few studies that report the quantification of phenolic compounds in the Brazilian noni fruit, however, with little data in the literature for possible comparisons between the fruit acquired in the Northeast region. In addition, most studies pay attention to the beneficial effects offered strictly by the fruit pulp, and not by the noni residues such as seeds and peel which can also provide beneficial biological functions.

This work aims to dehydrate the Brazilian noni pulp from the Northeast region by freeze-drying process and to characterize the seed, peel, fresh pulp and lyophilized pulp powder, regarding their physicochemical composition, carotenoids, chlorophylls, sugars and ascorbic acid contents. Furthermore, the influence of the application of different solvents on the extraction of phenolic compounds and their antioxidant capacity was evaluated, in order to seek new sources and clarifications about the bioactive potential of the noni fruit and its components for their possible benefits and new applications.

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2 Materials and methods

2.1 Analytical standards and reagents

HPLC grade methanol and acetonitrile were obtained from the company, Tedia. The reagents ethanol, aluminum chloride, sodium carbonate, potassium phosphate buffer, sodium citrate, ferrous sulfate, Folin-Ciocalteau phenol reagent; FRAP reagent; 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS); 6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid (Trolox); 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and the analytical standards such as apigenin ($C_{15}H_{10}O_5$), artepillin C ($C_{19}H_{24}O_3$), cinnamic acid ($C_9H_8O_2$), caffeic acid ($C_9H_8O_4$), ferulic acid ($C_{10}H_{10}O_4$), caffeic acid phenyl ester ($C_{17}H_{16}O_4$), gallic acid ($C_7H_6O_5$), biochanin A ($C_{16}H_{12}O_5$), catechin ($C_{15}H_{14}O_6$), epicatechin ($C_{15}H_{14}O_6$), ethyl gallate ($C_9H_{10}O_5$), kampheride ($C_{16}H_{12}O_6$), isoramnetin ($C_{16}H_{12}O_7$), acacetin ($C_{16}H_{12}O_5$), pinocembrin ($C_{15}H_{12}O_4$), narigenin ($C_{15}H_{12}O_5$), *p*-coumaric acid ($C_9H_8O_3$), kaempferol ($C_{15}H_{10}O_6$), quercetin-3-glycosylated ($C_{21}H_{20}O_{12}$), protocatechuic acid ($C_7H_6O_4$), chlorogenic acid ($C_{16}H_{18}O_9$), rutin ($C_{27}H_{30}O_{16}$), vanillin ($C_8H_8O_3$) and vanillic acid ($C_8H_8O_4$) were purchased from Sigma-Aldrich (Saint Louis, MO, USA).

2.2 Samples

The noni fruit was purchased from the municipal market in the city of Aracaju (Latitude: 10°54'19.7"S Longitude: 37°02'55.2"W), located in the state of Sergipe, Brazil. The fruits were selected according to their stage of maturation considered as "mature". Later, the fruit was washed and sanitized with sodium hypochlorite. Subsequently, the peel, pulp and seeds were separated; each part of the fruit was placed in separate polyethylene containers and stored under freezing at -18 ºC, for later elaboration of extracts and analysis. Samples were analyzed in triplicate.

2.3 Obtaining the lyophilized pulp powder

Noni pulp was placed in polyethylene trays for freezing in a vertical freezer at -18 ºC for 48 h. Later, the frozen sample was placed in a freeze dryer (Christ Alpha 1–2 LD Plus) using the following processing parameters: temperature of -54.9 ºC, pressure of 6.11 mbar and vacuum of 0.42 mbar for a period of 48 h. After freeze-drying, the samples were triturated. Later, the lyophilized powder was stored in amber flasks and maintained in a desiccator until further analysis.

2.4 Preparation of extracts

Noni extracts were obtained using different solvents: 70% ethyl alcohol, acetone, 80% acetone and water according to Costa et al. (2013), with modifications. For extraction, 4 g of the crushed sample of peel, seed and pulp *in natura* and 1 g of lyophilized pulp powder were weighed in a glass beaker and 20 mL of solvent were added. Subsequently, the beakers were placed in a sonicator for 1 h at 25 °C. The extracts were filtered on qualitative paper (Unifil - grammage 80 g/m^2).

2.5 Physico-chemical characterization

The determinations of moisture, ash, proteins, lipids, soluble solids, acidity, water activity and pH were carried out according to the methodologies reported by Instituto Adolfo Lutz (2008).

2.6 Total carotenoids and chlorophylls

The total carotenoids and chlorophylls contents determinations were based on the methodology presented by Lichtenthaler (1987). Absorbance readings were measured in a spectrophotometer (Molecular Devices, USA; SpectraMax M2) at wavelengths of 470 nm (total carotenoids), and at 647 and 663 nm for chlorophylls.

The concentrations of total carotenoids and chlorophyll contents were calculated according to the Equations 1, 2, 3.

$$Chlorophyll\ a\ (Ca) = \left[12.25 \times A_{663} - 2.79 \times A_{647}\right] \quad (1)$$

$$Chlorophyll\ b\ (Cb) = \left[21.50 \times A_{647} - 5.10 \times A_{663}\right] \quad (2)$$

$$Carotenoids(mg/g) = \left[1000 \times A_{470} - \left(1.82 \times Ca - 104.96 \times Cb\right)\right]/198] \quad (3).$$

Where: A= absorbance.

2.7 Total phenolics

The total phenolics contents were determined according to the method proposed by Correia et al. (2004). The samples had their absorbance measured in a spectrophotometer (Molecular Devices, USA; SpectraMax M2) at the wavelength of 725 nm. The calibration curve was constructed from the analysis of different concentrations of gallic acid (0.1 - 0.0008 mg/mL). Results were expressed in μg gallic acid (GAE)/g sample.

2.8 Total flavonoids

The total flavonoids contents were determined according to the methodology published by Andrade et al. (2017). The absorbance reading was measured in a spectrophotometer (Molecular Devices, USA; SpectraMax M2) at 427 nm. The total flavonoids content was determined using a standard curve constructed of quercetin (0.1 - 0.0008 mg/mL). Results were expressed in terms of μg quercetin/g sample.

2.9 Antioxidant activity

*ABTS*Assay*

The ABTS assay followed the methodology of Re et al. (1999). Absorbance was measured in a spectrophotometer (Molecular Devices, Sunnyvale, CA, USA; SpectraMax M2) at 734 nm. Antioxidant capacity was determined using calibration curves prepared for trolox (0 – 450 ppm) and ascorbic acid (25 – 350 ppm). Results were expressed as μM of Trolox/100 g sample (TEAC) and μM of ascorbic acid/100 g sample (AAEAC).

FRAP assay

The ferric reducing antioxidant power (FRAP) values were determined according to the procedure described by Benzie & Strain (1996). Absorbance readings were taken in a spectrophotometer (SpectraMax M2) at 593 nm. Antioxidant capacity was determined using calibration curves prepared for trolox (0 – 160 ppm) and ascorbic acid (0 – 160 ppm). Results were expressed as mmol Trolox/g sample and mmol ascorbic acid/g sample.

2.10 Identification and quantification of phenolic compounds using LC-MS system

The identification and quantification of flavonoids and phenolic acids were performed following the methodology proposed by Andrade et al. (2017), with modifications. A LC-MS/MS system was used having a triple quadrupole mass analyzer (Agilent 6490 Triple Quad LCMS/MS). SIM (Selected Ion Monitoring) mode was used to monitor ions, with electrospray ionization in negative mode. The column used was Ascentis Express F5 (150 x 2.1 mm, 2.7 µm particle; Sigma Aldrich). The mobile phase consisted of: Solution A (deionized water with 0.1% formic acid) and Solution B (acetonitrile with 0.1% formic acid). The flow rate was 0.2 mL/min at a temperature of 40 °C and an injection volume of 2 µL. The elution was performed in gradient mode, according to the following events: 0-1 min, 85% A; 1-9 min, 75% A; 9-16 min, 70% A; 16-23 min, 60% A; 23-25, 55% A; 25-28 min, 50% A; 28-33 min, 40% A; 33-37 min, 25% A; 37-38 min, 85% A. The calibration curves of the analytical standards of the phenolic compounds were constructed and the analysis was performed in triplicate.

2.11 Statistical analysis

Data analysis was performed using Assistant Software 7.7, through analysis of variance (ANOVA) using the Tukey test at 95%. The Multivariate statistical analysis and Pearson's correlation coefficients were determined using SAS software version 9.1.

3 Results and discussion

3.1 Physico-chemical characterization of fruit noni

The lyophilized pulp powder showed higher protein (9.21 g/100 g) and ash contents (5.73 g/100 g) when compared to the other components of the fruit. However as expected, the lyophilized pulp powder had low water activity, pH, acidity and moisture contents (Table 1). The use of lyophilization as a drying method for pulp reduced the moisture content of the fruit by 83.21%. Noni seed demonstrated superior quantification for lipids (7.18 g/100 g). Nascimento et al. (2018) reported lower values of lipids (0.79 ± 0.03%), ash (0.60 ± 0.01%) and acidity (0.28 ± 0.01%) for noni seed from Maranhão (Brazil). According to Jahurul et al. (2021), noni seeds may be a valuable new source of vegetable oil due to their nutritional properties and non-toxic nature.

Faria et al. (2014) in study with noni pulp collected in the city of Cuiabá, Brazil reported moisture content (90.66%), soluble solids content (9.2 ºBrix), ash content (0.66%) and titratable acidity (0.44 g of citric acid/100 g); these values were similar to those observed in the present study. Motshakeri & Ghazali (2015) reported that the soluble solids contents in the fruit vary between 8 to 10º Brix. Thus, when relating issues such as the chemical composition of noni, it is necessary to take into account aspects such as environmental conditions, including soil, geographic location, stage of maturation and climatic conditions.

The chlorophyll a and b contents in the various components of noni fruit are presented in Table 1. The lyophilized pulp powder showed the highest concentration of chlorophyll a (0.55 ± 0.01 mg/g) and chlorophyll b (11.44 mg/g). Noni contains kaempferol, a chlorophyll derivative, in its composition, which may explain its high chlorophyll content (Abou Assi et al., 2017). When comparing the various components of the noni fruit, the sample that presented the highest values of carotenoids was the lyophilized powder of the fruit pulp (28.33 µg of β-carotene/g), differing significantly in the pulp. Previous studies reported that the carotenoid content in noni seed varies from 0.63 – 1.06 mg/100 g for mature noni seed and 0.71 mg/100 g for immature noni seed (Jahurul et al., 2021). Palioto et al. (2015) reported a lower content of carotenoids in noni pulp (0.45 mg/100 g). Septembre-Malaterre et al. (2016) found 0.51 mg/100 g of carotenoids in lychee, 0.052 mg/100 g in pineapple and 1.92 mg/100 g in papaya Colombo cultivar. Thus, when compared to other fruits, noni constitutes a considerable higher amount of carotenoids.

The concentration of carotenoids in the lyophilized pulp powder was four times higher when compared to the fresh pulp. Thus, lyophilization is a cold drying method that guarantees maintenance of the physicochemical characteristics and good nutritional and sensorial quality of the product. In addition, lyophilization of the noni fruit was carried out under two conditions, a period of 48 hours and 24 hours, and it was found that the latter was sufficient to dry the food matrix. Thus, in terms of cost-benefit aspects, although the freeze-drying process is expensive yet a short time process of 1 hour justifies its economic viability.

Table 1. Physicochemical characteristics of noni pulp, seed, peel and lyophilized pulp powder.

Characteristics	Pulp	Seed	Peel	Lyophilized pulp powder
Ash (%)	0.60 ± 0.02^{C}	1.09 ± 0.01^{B}	0.76 ± 0.05^{C}	5.73 ± 0.09^{A}
Moisture (%)	88.98 ± 0.38^{A}	6.16 ± 0.08^{D}	88.22 ± 0.20^{B}	14.94 ± 0.21^{C}
Protein (%)	0.93 ± 0.04^{C}	5.80 ± 0.37^{B}	0.86 ± 0.08^{C}	9.21 ± 0.18^{A}
Lipids (%)	0.14 ± 0.03^{B}	7.18 ± 0.23^{A}	0.20 ± 0.01^{B}	0.29 ± 0.07^{B}
pH	5.04 ± 0.03^{B}	6.16 ± 0.02^{A}	5.11 ± 0.03^{B}	3.90 ± 0.01^{C}
Acidity (g of citric acid/100 g)	0.44 ± 0.01^{A}	0.44 ± 0.02^{A}	0.44 ± 0.03^{A}	0.35 ± 0.02^{A}
Total soluble solids (°Brix)	9.06 ± 0.05^{A}	-	8.50 ± 0.00^{B}	--
A_w	0.92 ± 0.00^{A}	0.36 ± 0.00^{B}	0.91 ± 0.00^{A}	0.35 ± 0.00^{B}
Chlorophyll a (mg/g)	0.42 ± 0.00^{A}	0.40 ± 0.01^{A}	0.41 ± 0.00^{A}	0.55 ± 0.01^{A}
Chlorophyll b (mg/g)	0.74 ± 0.02^{B}	0.67 ± 0.01^{B}	0.67 ± 0.01^{B}	11.44 ± 0.29^{A}
Total carotenoids (µg of β-carotene/g)	6.66 ± 0.15^{B}	5.71 ± 0.05^{C}	6.49 ± 0.06^{B}	28.32 ± 0.10^{A}

Means followed by the same capital letters on the same line do not differ statistically ($p < 0.05$) from each other by applying the Tukey test.

3.2 Sugars and organic acids

The sugars glucose, fructose and sucrose were detected in the lyophilized pulp powder of noni in higher concentrations, with glucose being the major constituent (9.21 mg/100 g), differing significantly ($p < 0.05$) from the fresh pulp, peel and seed (Table 2). In addition, when comparing the peel and pulp, it was possible to notice that there was no statistical difference ($p < 0.05$) and that none of the investigated sugars was detected in the noni seed. Studies on the specific quantification of sugars present in noni are little reported. Dussossoy et al. (2016) identified and quantified glucose (2.07 g/100 g) and fructose (2.44 g/100 g), showing lower values. Beltrão et al. (2014) reported that the total sugar content of noni was 5.88 g/100 g, with 4.88 g/100 g represented by reducing sugars (glucose and fructose).

The ascorbic acid content determined by liquid chromatography (Table 2) ensured better results of ascorbic acid for the lyophilized powder (336.62 ± 0.00 mg/100 g), with a value 2.5 times higher than that found in fresh pulp (130.04 ± 0.00 mg/100 g). In this sense, the freeze-drying process is a viable method for better conservation of vitamin C.

Ruhomally et al. (2015) reported that the ascorbic acid content of the freeze-dried mature noni was 76.24 ± 1.13 mg/100 g. Costa et al. (2013) reported lower amounts of 23.1 mg/100 g. Previous studies reported that the vitamin C content of noni seed ranges from 94.0–136 mg/100 g for mature noni seed and 126.8 mg/100 g for immature noni seed (Jahurul et al., 2021). Variations in values for vitamin C are directly correlated to the degree of maturation of the fruit, since during the maturation period there was a decrease in this compound. Furthermore, Silva et al. (2022) reported that the L-ascorbic acid content of acerola peel was 3.06 ± 0.04 $mg.g^{-1}$ of sample on dry basis; this content is similar to the content found in the lyophilized pulp powder of noni 336.62 ± 0.00 mg/100 g, suggesting that the noni fruit could be considered a fruit with a relevant amount of vitamin C.

3.3 Total phenolics and flavonoids

The contents of total phenolics and flavonoids present in the pulp, peel, seed and lyophilized pulp powder of noni showed a significant difference ($p < 0.05$) between the different extraction solvents (Figure 1A). The phenolic content varied from 199.64 ± 3.77 to 7486.38 ± 5.65 µg GAE/g. It is observed that the lyophilized pulp powder presented significantly higher contents in the aqueous (7486.3770 µg GAE/g sample) and 70% ethanol (4906.2250 µg GAE/g sample) extracts when compared to the other components of the fruit. The noni seed exhibited higher values in the extracts with the solvents acetone and acetone 80%, with values of 2329.9920 and 1539.1470 µg GAE/g sample, respectively.

Ruhomally et al. (2015) quantified total phenolics in noni extracts and reported values of 748.40 µg and 770.34 µg for ripe and unripe fruits, respectively. Krishnaiah et al. (2015) in a methanolic extract of the seed using a high pressure reactor found a value of 43.18 mg GAE/10g in sample for total phenolics.

The total flavonoids content varied from 23.11 ± 0.05 to 385.57 ± 2.52 µg QE/g sample. The lyophilized pulp powder presented better results when compared to the peel, seed and fresh pulp in the aqueous extracts, 80% acetone and 70% ethanol, varying from 263.76 ± 2.07 to 385.57 ± 2.52 µg QE/g sample. The seed extract, obtained with acetone had the value of 156.94 µg QE/g sample, presenting higher value in relation to the other extracts. It is therefore evident that the type of solvent for the extraction of phenolics and flavonoids influences the final results of the bioactives present in noni.

3.4 Antioxidant capacity

The *in vitro* assays demonstrated the antioxidant capacity of noni which are presented in Table 3. The antioxidant activity applied to the $ABTS^{+}$ radical scavenging method varied from 26.60 ± 0.00 to 3381.15 ± 15.96 mmol Trolox/100 g (Equivalent to Trolox) and 137.46 ± 0.87 to 3339.59 ± 35.38 mmol AA/100 g (Ascorbic Acid Equivalent). It was observed that the lyophilized pulp powder obtained a more expressive result (725.34 ± 35.83 to 3381.15 ± 15.96 mmol/100 g) when compared to the other noni samples. When comparing the performance with different solvents, the lyophilized pulp powder had better antioxidant capacity when extracted with 70% ethanol and the seed and peel had a more satisfactory response with 80% acetone. Gironés-Vilaplan et al. (2014) reported a lower value (9.04 mmol trolox/100g) for the antioxidant activity of noni fruit.

The antioxidant activity by the FRAP method varied from 5535.31 ± 37.48 to 312014.20 ± 2397.19 mmol TE /100 g (equivalent to Trolox) and 129785.30 ± 2397.19 to 467970.40 ± 5085.25 mmol AA/100 g (equivalent to ascorbic acid), with emphasis on the lyophilized pulp powder presenting the highest antioxidant activity among the aqueous extracts being 312014.20 ± 2397.19 mmol TE/100g and 467970.40 ± 5085.25 mmol. AA/100g, followed by ethanolic extracts 70%, acetone 80% and acetone.

Table 2. Sugars and organic acids contents in pulp, seed, peel of noni and lyophilized pulp powder.

	Lyophilized pulp powder	Pulp	Peel	Seed
Sugars				
Sucrose (g/100 g)	4.69 ± 0.32^{cA}	3.18 ± 0.29^{bB}	3.30 ± 0.24^{aB}	nd
Fructose (g/100 g)	6.38 ± 0.37^{bA}	3.19 ± 0.23^{bB}	3.14 ± 0.88^{aB}	nd
Glucose (g/100 g)	9.21 ± 0.69^{aA}	4.81 ± 0.48^{aB}	3.92 ± 0.43^{aB}	nd
Organic acids				
Ascorbic acid (mg/100 g)	336.62 ± 0.00	130.04 ± 0.00	142.44 ± 0.00	nd

Means followed by the same lowercase letters on the same line do not differ statistically ($p < 0.05$) from each other by applying Tukey's test. Means followed by the same capital letters in the same column do not differ statistically ($p < 0.05$) from each other by applying the Tukey test. nd: Not detected.

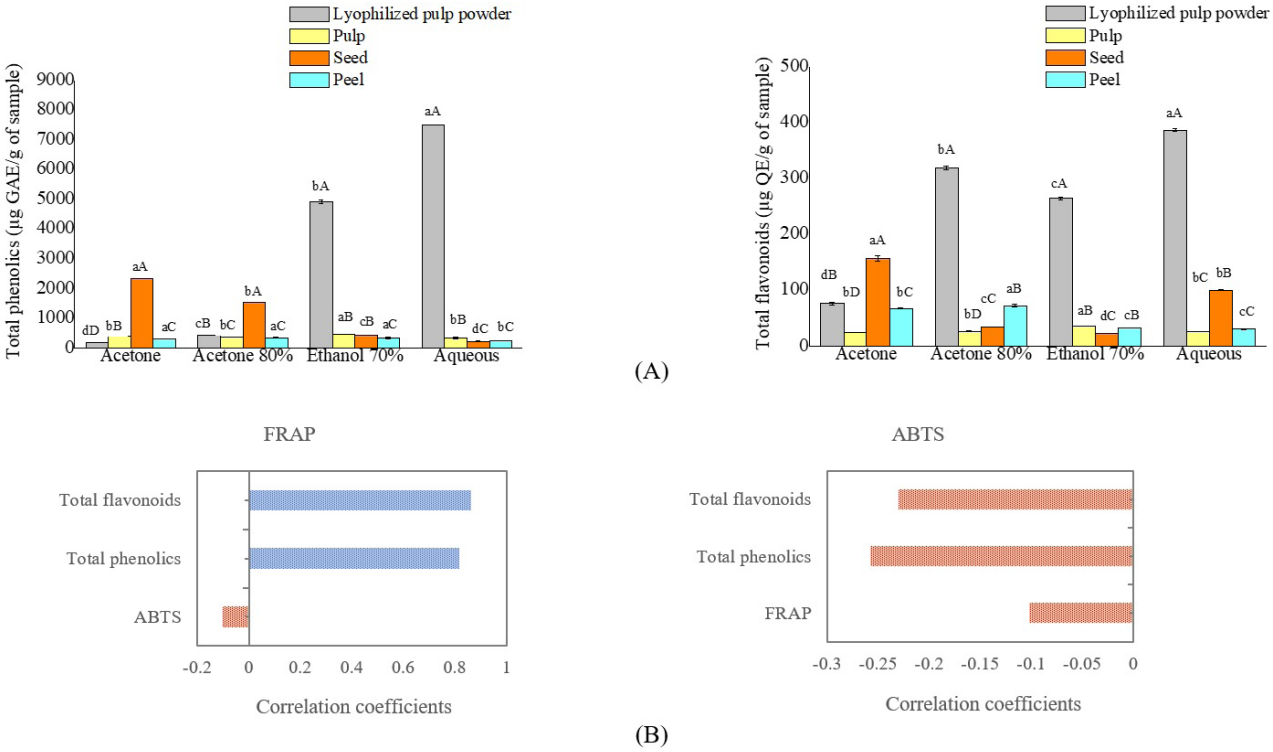

Figure 1. Total phenolics and flavonoids contents present in pulp, seed, peel of noni and lyophilized pulp powder (A) and correlation of antioxidant activity between FRAP and ABTS (B).

Table 3. Antioxidant activity (ABTS and FRAP) in different extracts of pulp, seed, peel of noni and lyophilized pulp powder.

Sample / Method	Trolox (Mmol Trolox/100 g)				Ascorbic acid (Mmol. AA/100 g)			
	Acetone	Acetone 80%	Ethanol 70%	Aqueous	Acetone	Acetone 80%	Ethanol 70%	Aqueous
	ABTS ($ABTS^{+}$ free radical capture)							
Lyophilized pulp powder	725.34 ± 35.83^{aF}	3093.64 ± 55.44^{aC}	3381.15 ± 15.96^{aA}	2626.12 ± 23.90^{aE}	759.53 ± 31.82^{aF}	2862.47 ± 49.24^{aD}	3284.76 ± 7.02^{aB}	3339.59 ± 35.38^{aA}
Pulp	144.27 ± 0.20^{bE}	114.70 ± 0.60^{cE}	64.76 ± 0.60^{cF}	53.77 ± 0.40 ^{bF}	649.77 ± 0.86^{bA}	536.07 ± 2.65^{cB}	315.59 ± 2.65^{cC}	259.80 ± 1.72^{bD}
Seed	42.19 ± 2.39^{cF}	172.04 ±1.99^{bDE}	136.08 ± 0.00^{bE}	45.58 ± 4.19^{bF}	215.79 ± 10.62^{dCD}	833.80 ± 2.66^{bA}	632.57 ± 0.00^{bB}	231.26 ± 18.62^{bC}
Peel	72.15 ± 1.60^{cDE}	90.53 ± 0.00^{cD}	24.81 ± 0.20^{dF}	26.60 ±0.00^{bEF}	336.40 ± 6.83^{cB}	424.91 ± 0.00^{dA}	137.46 ± 0.87^{dC}	143.12 ± 0.00^{cC}
	FRAP (Ferric Reducing *Antioxidant* Power)							
Lyophilized pulp powder	86537.46 ± 0.00^{aF}	10360.39 ± 0.00^{bH}	123384.60 ± 700.77^{bE}	312014.20 ± 2397.19^{aB}	129785.30 ± 0.00^{aD}	15539.58 ± 0.00^{bG}	185055.80 ± 1051.15^{bC}	467970.40 ± 5085.25^{aA}
Pulp	9881.29 ± 34.84^{bB}	10676.30 ± 20.74^{bB}	6323.12 ± 0.00^{cC}	11434.31 ± 8.08^{bB}	14821.69 ± 52.26^{bA}	16014.18 ± 31.16^{bA}	9484.43 ± 0.00^{cB}	17151.22 ± 12.13^{bA}
Seed	9657.53 ± 54.04^{bF}	25973.12 ± 87.41^{aD}	144490.80 ± 262.21^{aB}	5535.31 ± 37.48^{cG}	14486.05 ± 81.06^{bE}	38954.44 ± 131.15^{aC}	216731.00 ± 93.31^{aA}	8302.72 ± 56.22^{cFG}
Peel	8894.37 ± 11.23^{bBC}	9266.06 ± 54.17^{bBC}	5835.02 ± 61.76^{cD}	6925.24 ± 24.32^{cCD}	13341.31 ± 16.85^{bA}	14184.87 ± 81.25^{bA}	8752.28 ± 92.64^{cBC}	10387.62 ± 36.49^{cB}

Means followed by the same lowercase letters on the same column do not differ statistically ($p < 0.05$) from each other by applying Tukey's test. Means followed by the same capital letters in the same line do not differ statistically ($p < 0.05$) from each other by applying the Tukey test.

Nascimento et al. (2018) reported lower values for antioxidant activity by the FRAP method for pulp (38.07 ± 0 µM $TE.g^{-1}$), peel (57.95 ± 1.82 µM $TE.g^{-1}$) and seed (34.79 ± 0.95 µM $TE.g^{-1}$) of noni from the city of Maranhão, Brazil. Lower values were reported by Ruhomally et al. (2015) when determining the ferric chloride reduction potential for green (11.90 mmol/g) and ripe (11.26 mmol/g) noni fruit. Gironés-Vilaplan et al. (2014) reported a value of 6.92 mmol/100 g for antioxidant activity in noni pulp. Thus, this study showed high antioxidant potential in noni fruit, highlighting mainly the positive effect caused by the freeze-drying process.

According to Gironés-Vilaplan et al. (2014), noni has good antioxidant capacity due to the considerable presence of flavonoids, in particular, kaempferol. The extracts show that unripe fruits have a profile with greater potential for reduction and chelating iron; on the other hand, the ripe fruit works as a scavenger of nitric oxide and superoxide radicals.

Figure 1B shows Pearson's correlation between antioxidant activity with phenolics and total flavonoids. A higher and better correlation of phenolics and flavonoids was observed for noni by the FRAP antioxidant assay. This is because it presented

strong and positive coefficients between phenolics (0.8160) and flavonoids (0.8630), being significant at the 1% probability level ($p < 0.01$). Antioxidant activity by the ABTS method did not show significant results. In this sense, the antioxidant compounds present in the noni extracts did not show efficient interaction with the $ABTS^{+}$ radical due to their low reactivity between them.

3.5 Identification and quantification of phenolic compounds by LC-MS

Table 4 presents the contents of phenolic compounds present in noni fruit. The phenolic compounds artepillin C, biochanin A, caffeic acid, eriodictyol, isorhamnetin, kaempferide, kaempferol, narigenin, pinocembrin, ferulic acid, quercetin-3-glucoside, rutin, vanillin and vanillic acid were quantified in the extracts of the noni fruit parts, totaling the content of phenolic compounds quantified as 4282.30 μg/100 g (peel), 1320.00 μg/100 g (pulp) and 589.40 μg/100 g (seed). Furthermore, it is observed that the compounds isorhamnetin and pinocembrin were determined only in the extract of noni peel, suggesting that it is a marker for this residue.

Among the various compounds, rutin was reported as the major flavonoid, found predominantly in the peel and pulp of the fruit with values of 4060 μg/100 g and 1290 μg/100 g, respectively. Rutin is one of the main therapeutically bioactive constituents and has antioxidant, antidiabetic, anticancer, anti-allergic, anti-inflammatory, cardioprotective properties (Semwal et al., 2021).

In noni seeds, the major flavonoids were caffeic acid (496 μg/100 g), vanillin (36 μg/100 g) and vanillic acid (21 μg/100 g). However, no studies were reported in the literature regarding the quantification of these compounds in noni seed. Caffeic acid has beneficial activities such as antitumor, anti-inflammatory, antioxidant and antiviral (Hernández-Chávez et al., 2018).

Table 4. Phenolic compounds contents in pulp, seed, peel of noni by LC-MS.

Phenolic compounds	Contents (μg/100 g)		
	Peel	Seed	Pulp
Artepillin C	45.00	3.00	18.00
Biochanin A	7.00	0.40	nd
Caffeic acid	1.00	496.00	nd
Eriodictyol	1.00	2.00	nd
Isorhamnetin	6.00	nd	nd
Kaempferide	11.00	5.00	6.00
Kaempferol	28.00	2.00	3.00
Narigenin	0.30	1.00	nd
Pinocembrin	2.00	nd	nd
Ferulic acid	3.00	2.00	1.00
Quercetin-3-Glucoside	93.00	3.00	1.00
Rutin	4060.00	18.00	1290.00
Vanillin	1.00	36.00	0.10
Vanillic acid	24.00	21.00	0.90
∑ *Phenolics compounds*	4282.30	589.40	1320.00

nd = Not detected.

According to Wang et al. (2022), vanillin reduces hippocampal neuronal death in mouse models of global cerebral ischemia. Vanillic acid has antioxidant, anticancer, antidiabetic, anti-inflammatory, antiobesity and antibacterial effects (Kaur et al., 2022).

In addition, higher amounts of artepillin C (18 μg/100 g) and kaempferide (6 μg/100 g) were also reported in the noni pulp. Artepillin C includes beneficial health pharmacological effects such as antioxidants, anticancer, anti-inflammatory, antidiabetic, immunomodulatory, neuroprotective, gastroprotective and antimicrobial (Shahinozzaman et al., 2020).

In the ethanolic extracts of noni leaves, Bonechi et al. (2018) ensured a positive effect when evaluating the protective capacity of quercetin and rutin on induced oxidative stress. Quercetin is also beneficial, offering improvements in the human body, appearing as a lipophilic compound having functional characteristics, well known as the most abundant of flavonoids in food sources, ensuring an effect against obesity mediated by the AMPK and MAPK signaling pathways (Bigliardi & Galati, 2013). Moreover, Ruhomally et al. (2015) suggest that noni fruit may be an interesting source of prophylactic antioxidants modulated by the polyphenol composition.

The freeze-drying process gave good results, being a favorable method for the maintenance and conservation of the fruit's characteristics. The lyophilized powder of noni pulp contained high levels of carotenoids, chlorophyll, and high contents of phenolics and flavonoids when compared to the other components of the fruit. In addition, noni has a high level of antioxidant activity *in vitro* by the FRAP method. The high content of ascorbic acid makes the fruit an excellent source of vitamin C, especially in the freeze-dried pulp powder, which is approximately double in content when compared to fresh pulp. Thus, noni presents promising bioactive components for a good antioxidant capacity. However, more studies should be undertaken to determine the efficiency and safe dosage to maintain its health benefits.

Conflict of interest

All authors declare that they have no conflict of interest.

Availability of data and material

Not applicable.

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Author contributions

RFF – Conceptualization, Formal analysis, Project administration, Writing – original draft; JKSA – Investigation and Formal analysis, MR – Phenolic compounds identification and quantification and NN – Fund resources, Writing – Review and editing and Supervision.

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