GREEN CHEMISTRY: CHARACTERIZATION OF BIOACTIVE COMPOUNDS PRESENT IN THE PEEL OF RAMBUTAN (*Nephelium lappaceum L.*) EXTRACTED IN AZEOTROPIC SOLUTION.

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ABSTRACT

Nephelium lappaceum L., known as Rambutan, is a tropical fruit rich in bioactive compounds and alkaloids. The objective of this work was to reuse and add value to this residue, from the characterization of the bioactive compounds extracted from an azeotropic extractor solution (ethanol: distilled water: acetic acid in the ratio 1:8:3 v/v/v), using green chemistry. The content of flavonoids (1,45-2,09 mg QE/g Extract), phenolic content (4.37-5.43 mg GAE/g Extract), condensed tannins (1,73-4,47 µg EC/g Extract), carotenoids (228 and 89 β-carotene and lycopene µg/100g Extract, respectively), anthocyanins (282,21 mg/100g Extract) and antioxidant activity (5,29-6,47 µM TE/g Extract for the FRAP reagent; inhibition of 34,23-42,76% for the ABTS radical and 57,34-73,41% for the DPPH radical) of the extracts were evaluated. According to the results obtained, rambutan peel extracts are rich in anthocyanins and have a high potential for inhibition and/or reduction of oxidative reactions, a characteristic that enables their application as antioxidant agents and/or natural colorants in the various segments of the cosmetic, food and pharmaceutical industries.

Keywords: *Nephelium lappaceum* L. 1. Bioactive compounds 2. Extractor solution 3. Residue 4. Green chemistry 5.

1 INTRODUCTION

Nephelium lappaceum L., known as Rambutan, belonging to the Sapindaceae family, is highly appreciated for its exotic appearance and pleasant flavor, which has led to an increase in its commercialization and processing around the world. However, only 52% of the weight of the fruit is edible, the rest, which corresponds to peels and seeds, are discarded, which can generate a high volume of waste and economic losses.

The objective of this study was to contribute to the existing knowledge in the area of use of rambutan peel in the cosmetic, pharmaceutical and other industries, as a way to reuse and add value to this waste, from the characterization of the bioactive compounds extracted from an innovative extractor solution, aiming at a cleaner and more economical process.

2 MATERIAL & METHODS

Fruits of Nephelium lappaceum L. (Rambutan), in the mature stage, were acquired in Santa Bárbara/PA and, after botanical identification, the peels (exocarp) were removed and washed in running water. The extractor solution was obtained through the ethanol:distilled water:acetic acid solution in the ratio 1:8:3 v/v/v. For the preparation of the extract, the ratio of 10:6 v/m of solution:peels was used and 3 variations of the extract were obtained for study: maceration for 7 days (RBT 7D), maceration for 24 hours (RBT 24H) and exhaustive extraction (RBT EEX). The 3 variations under study underwent column chromatography to quantitatively analyze the bioactive compounds of the mixtures. The adsorbent chosen for the process was silica and the approximate ratio of the adsorbent (g)/sample (g) was 20:1, for the packing of the column the dry packing method was adopted. Because the liquid samples were placed directly on the stationary phase without the need for dilution, 3 solvents were passed, the first with acetone, the second with ethanol and the third with distilled water.

All analyses were performed in triplicate in a UV-Vis 1800 spectrophotometer (Shimadzu®, Kyoto, Japan):

For the determination of antioxidant activity by ABTS free radical capture, the radical was prepared from the reaction of 5 mL of ABTS stock solution (7 mM) with 88 µL of potassium persulfate solution (140 mM) and was kept in the dark and at room temperature for 16 hours. Subsequently, 1 mL of the mixture was diluted in ethyl alcohol until an absorbance of 0.7 nm ± 0.05 nm at 734 nm was obtained. Then, a standard trolox curve (2,000 µM) was constructed with concentrations ranging from 100 µM to 2,000 µM. In a dark environment, an aliquot of 30 µL of each trolox solution (100 µM, 500 µM, 1000 µM, 1500 µM and 2,000 µM) was transferred to test tubes and 3.0 mL of the ABTS radical solution was mixed. Then, the diluted mixtures were vortexed and after 6 min of the mixture, the readings were performed at 734nm. Ethyl alcohol was used as white alcohol to calibrate the

spectrophotometer. To determine the antioxidant activity in the sample, the same procedure as above was repeated with the dilution of the extract. The final concentration expressed in µM of Trolox per gram of sample[12,13].

For the determination of antioxidant activity by DPPH free radical capture, DPPH radical solution was prepared from 24 mg of DPPH in 100 mL of ethanol. Subsequently, 10 mL of the solution was removed and transferred to a 100 mL volumetric flask and the volume was completed with ethanol to obtain the working solution. In a dark environment an aliquot of 150 µL of the sample was transferred to test tubes, mixed with 5,085 mL of the DPPH radical of the working solution, and homogenized in vortex. After 30 minutes of reaction, the reading was performed at a wavelength of 515 nm. Ethyl alcohol was used as white to calibrate the spectrophotometer. Antioxidant activity was calculated based on a standard Trolox curve (50µM-1000 µM). The final concentration expressed in µM of Trolox per gram of sample[15].

For the determination of antioxidant activity by the iron reduction method (FRAP), the FRAP reagent was obtained from the combination of 100 mL of 0.3 M acetate buffer, 10 mL of a 10 mM TPTZ solution, and 10 mL of a 20 mM ferric chloride aqueous solution, used immediately after preparation. In a dark environment, an aliquot of 90 µL was transferred from the solution of the extraction of phenolic compounds to test tubes, 270 µL of distilled water, 2.7 mL of FRAP reagent, homogenized in vortex and kept in a water bath at 37 °C for 30 minutes. Then, the reading was performed at a wavelength of 595 nm. The FRAP reagent was used as blank to calibrate the spectrophotometer. Antioxidant activity was calculated based on a standard Trolox curve (160 µM-1,600 µM). The final concentration expressed in µM of Trolox per gram of sample [2,14].

A standard gallic acid curve was constructed for the quantification of total polyphenol (TPC) content at concentrations of 5 to 75 mg/mL. In a 10 mL volumetric flask, 500 µL of Folin-Ciocalteu reagent were added, 6 mL of distilled water was added, after 2 min, 2 mL of sodium carbonate solution at 20% (w/v) were added to the solution and agitation was performed for 30 seconds, completing the volume with distilled water. The solutions remained at rest for 2 hours, then readings were taken at the wavelength of 760 nm. The same procedure is repeated for the samples, with an addition of 100 µL of extracts. The results were expressed in milligrams of gallic acid equivalents per gram of sample[5].

A standard quercetin curve was constructed for the quantification of total flavonoid content (TFC) at concentrations of 5 to 30 mg/mL. In a 10 mL volumetric flask, the different concentrations of the standard were added and 1 mL of 2.5 % (w/V) aluminum chloride solution was added. The solutions remained at rest for 30 min, then the readings were performed in a spectrophotometer at a wavelength of 425 nm. To determine the flavonoid content in the sample, 800 µL of the sample, 1 mL of 2.5% aluminum chloride solution (w/V), q.s.p ethanol were used. The results were expressed in milligrams of quercetin per gram of sample[5].

The concentration of condensed tannins (CT) in the extracts was performed by the reaction of 1% vanillin and 8% hydrochloric acid (HCl). A standard curve was constructed at concentrations from 20 to 100 /mL using catechin as standard. In 25 mL test tubes, 5 ml of the vanillin-HCl mixture 1:1 (in triplicate) was added. The tubes were preheated in a water bath at 30 ºC for 30 min. Then, the different concentrations of catechin were added and vortexed for 30 seconds. The reaction was maintained at 30º C for 30 minutes. The readings were taken at 500 nm, within a maximum period of 1 hour. The same procedure described above was repeated to determine tannins in the samples, with the addition of 1 mL of the sample. The results were expressed in micrograms of catechin per gram of the extract[1,3].

The carotenoids β-carotene (β-C) and lycopene (LCP) were analyzed in a dark environment, where 5 g of the extract was weighed and 25 mL of ice-cold acetone (T < 8 °C) was added with a volumetric pipette. Subsequently, the mixture was stirred gently and stored in the refrigerator for 2 h. After this period, the sample was stirred for 10 min at low temperature. After separation, the supernatant was partitioned to petroleum ether and successive washes were performed to remove the acetone. Subsequently, the sample was saponified in methanol and stored in a dark environment for 2 h at room temperature, then stirred for 30 min at low temperature for a new partition in petroleum ether and washing. Residual water was removed with the addition of anhydrous sodium sulfate (10 to 15 g). Petroleum ether was used as white to calibrate the spectrophotometer[9].

The total amount of anthocyanins monomeric (TAM) was determined using the adapted differential pH method[6]. According to the method, the difference in absorbance of pH 1.0 and 4.5 solutions is directly proportional to the TMA concentration. After dilution of the anthocyanin extract, the volumes of the anthocyanin-containing solutions are increased with the respective buffer solutions. The absorbance of buffered samples at the two different pHs is determined at the lengths of 510 nm and 700 nm. The results were expressed in milligrams of anthocyanin per 100 grams of extract.

3 RESULTS & DISCUSSION

The results obtained from the analyses performed on the extracts are shown in Table 1.

Table 1 Flavonoid content, phenolic content, condensed tannins, carotenoids, anthocyanins and antioxidant activity present in Nephelium lappaceum peel extracts.

Extract	TFC [a]	TPC [b]	CT [c]	β-C [d]	LCP [d]	TAM [e]	FRAP [f]	ABTS In[h]	DPPH In[h]
RBT 7D	2,09	4,37	1,73	-	-	282,21	5,29	42,76	57,34
RBT 24H	1,45	5,12	-	-	-	-	6,47	39,91	73,41
RBT EEX	1,92	5,43	4,47	228	89	-	6,33	34,23	60,59

Maran et al. (2017)	33,46[i]	167,58		-	-	3,17	-	-	-
Torgbo et al. (2022)	-	-	-	-	-	-	5980,35[j]	-	-
Oliveira et al. (2014)	-	2,56[j]	-	-	-	-	-	-	-

Legend: [a] (mg QE/g Extract); [b] (mg GAE/g Extract); [c] (µg EC/g Extract); [d] (µg/100g Extract); [e] (mg/100g Extract); [f] (µM TE/g Extract); [g] (mg/L); [h] (%); [i] (mg RE/100 g); [j] (g GAE/g Extract).

Many factors, such as solvent composition, extraction time, extraction temperature, and solid solvent ratio, can significantly influence extraction efficiency and yield. For the extraction of polyphenols, a range of solvents are used, from polar to nonpolar solvent mixtures. Some studies indicate that the ideal temperature for the extraction of polyphenols is in the range of 80°C, since at this temperature there is a large amount of polyphenols extracted, although their biological activity is maintained[8]. However, as this study was focused on extraction analysis aiming at green chemistry, the extractor solution was elaborated with a high proportion of water, with low toxicity and without the use of heat sources, it is also known that water, due to its polarity, efficiently extracts antioxidant compounds. The discussion is proven by analyzing the results obtained experimentally.
Condensed tannins are made up of flavonoid units, which are very resistant to microbiological degradation and currently have a greater commercial relevance, due to their greater availability[7]. Carotenoids have as one of their most important functionalities, the antioxidant, an advantage especially when obtained from waste, such as rambutan peel, which can be a great attraction for industries No comparative results were found in the literature regarding condensed tannins and carotenoids for the matrix studied, however, the values obtained for carotenoids are close to those of watermelon (141 and 71 µg/100g of β-carotene and lycopene, respectively)[4].

4 CONCLUSION

According to the results obtained, rambutan peel extracts are rich in anthocyanins and have a high potential for inhibition and/or reduction of oxidative reactions, a characteristic that enables their application as antioxidant agents and/or natural colorants in the various segments of the cosmetic, food and pharmaceutical industries.

REFERENCES

[1] AGOSTINI-COSTA, T. S.; GARRUTI, D. S.; LIMA, L.; FREIRE, S.; ABREU, F. A. P.; FEITOSA, T. Evaluation of methodologies for determination of tannins in cashew juice. CEPPA Bulletin, Curitiba, v. 17, n. 2, p. 167-176, 1999.

[2] BENZIE, FF; STRAIN, J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry, v. 239, n. 1, p. 70-76, 1996.

[3] BROADHURST, R. B.; JONES, W. T. Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture, Baffins Lane Chichester, v. 29, n.9, p. 788-794, 1978.

[4] DIAS, M.G.; BORGE, G.I.A.; KLJAK, K.; MANDI´C, A.I.; MAPELLI-BRAHM, P.; OLMEDILLA-ALONSO, B.; PINTEA, A.M.; RAVASCO, F.; TOMBBAS ŠAPONJAC, V.; SEREIKAITE, J.; ET AL. Ėuropean Database of Carotenoid Levels in Foods. Factors Affecting Carotenoid Content. Foods 2021, 10, 912. https://doi.org/10.3390/ foods10050912.

[5] FUNARI, C.S. & FERRO, V.O., 2006. Propolis analysis. Ciênc. Technol. Feed. 26(1):171-178.

[6] GIUSTI, M. M.; WROLSTAD, R. E. Characterization and measurement of anthocyanins by'UV-Visible spectroscopy. Current Protocols in Food Analytical Chemistry, F1.2.1-F1.2.13,2001.

[7] JORDAAN, M. The analysis of natural and sulfited commercial quebracho (Schinopsis Lorentzii) and Acacia (Acacia Mearnsii) proathocyanidin extracts with electrospray ionization mass spectrometry, 2013.

[8] LEON, M. F. M.; DUARTE, J. D.; SCHMITT, E. G. S.; QUINTANA, L. D.; ZAMBRANO, L. A. B.; ROCHA, M. B.; ZURAVSKI, L.; OLIVEIRA, L. F. S.; MACHADO, M. M. Evaluation of the efficiency of polyphenol extraction from tea samples using domestic methods. Electronic Journal of Pharmacy, vol. XIII, n. 2, p. 82-88, 2016.

[9] MALDONADE, I. R.; LOZADA, M. I. O.; OLIVEIRA, L. de L. de; The objective of this work was to establish a method for the quantification of total carotenoids by UV-Vis spectrophotometry and β-carotene by high performance liquid chromatography in oil samples. Embrapa Vegetables. 2021.

[10] MARAN, J. P.; MANIKANDAN, S.; NIVETHA, C. V.; DINESH, R. Ultrasound assisted extraction of bioactive compounds from Nephelium lappaceum L. fruit peel using central composite face centered response surface design. Arabian Journal of Chemistry. 2017.

[11] OLIVEIRA, H. A.; CAMPOS, C. S.; VAREJÃO, M. J. C.; NASCIMENTO, C. S. Determination of total polyphenols and antioxidant activity of agro-industrial residues from the Amazon. ISSN 2178 9665. 2014.

[12] RE, R; PELLEGRINI, N ET AL. Trolox ASSAY. Int. Antioxid. Res. Centre, Guy's, King's St Thomas' Sch. Biomed. Sci. Kings Coll. Campus London SE1 9RT UK 26. Free Radic. Biol. Med. 1999, 26, 1231–1237, doi:10.1016/s0891-5849(98)00315-3.

[13] RUFINO, M. do S. M.; ALVES, R. E.; BRITO, E. S. de; MORAIS, S. M. de; SAMPAIO, C. de G.; PÉREZ-JIMÉNEZ, J.; SAURA-CALIXTO, F. D. Scientific methodology: determination of total antioxidant activity in fruits by ABTSº+ free radical capture. 2007.

[14] RUFINO, M. S. M.; ALVES, R. AND ET AL. Scientific methodology: determination of total antioxidant activity in fruits by the iron reduction method (FRAP). Fortaleza: Embrapa Tropical Agroindustry, 2006. 4 p. (Technical Communiqué).

[15] SILVEIRA, A. C.; KASSUIA, Y. S.; DOMAHOVSKI, R. C.; LAZZAROTTO M (2018) Adapted DPPH method: a tool to analyze antioxidant activity of yerba mate fruit pulp in a fast and reproducible way. Comun Técnico 421 – Embrapa, 2018.

[16] TORGBO, S; RUGTHAWORN, P; SUKATTA, U; AND SUKYAI. P. Biological Characterization and Quantification of Rambutan (Nephelium lappaceum L.) Peel Extract as a Potential Source of Valuable Minerals and Ellagitannins for Industrial Applications. Selorm Torgbo, Prapassorn Rugthaworn, Udomlak Sukatta, and Prakit Sukyai. ACS Omega 2022 7 (38), 34647-34656. DOI: 10.1021/acsomega.2c04646.

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