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Pharmacognostic study and evaluation of the antioxidant capacity of the fruit of two varieties of *Nephelium lappaceum* L. (*Sapindaceae*), (rambutan)

[Estudio farmacognóstico y evaluación de la capacidad antioxidante de los frutos de dos variedades de *Nephelium lappaceum* L. (rambutan)]

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Abstract

Context: *Nephelium lappaceum* (achotillo or rambutam), is a fruit native to Malaysia and Indonesia introduced in Ecuador for consumption purposes.

Aims: To identify the bioactive metabolites present in the peels and seeds of the sweet and bitter varieties of rambutan fruits and their antioxidant capacity.

Methods: It was performed through a pharmacognostic analysis of the peels of the fruits, based on the evaluation of the physicochemical parameters, the phytochemical analysis, the quantification of the phenols and flavonoids, the determination of the antioxidant capacity and the identification of the fatty compounds present in the seeds by gas chromatography coupled to mass spectrometry.

Results: The results showed that both the peels and the seeds of the two varieties differ in the content of phyto-constituents and antioxidant properties.

Conclusions: The bitter fruit presented a higher concentration of phenols and flavonoids in the peels and a higher concentration of fatty compounds in the seeds, with marked differences between the fractions of the non-saponifiable compounds. For the fatty acid fraction, a greater qualitative similarity was evidenced, with quantitative differences**.** Although the peels extract of both fruits have antioxidant capacity, it was significantly higher in bitter fruits.

Keywords: antioxidant; fatty compounds; fruit peels; phenolic compounds; seeds.

Resumen

Contexto: *Nepheliumlappaceum* (achotillo o rambutam), es una fruta originaria de Malasia e Indonesia introducida en Ecuador con fines de consumo.

Objetivos: Identificar los metabolitos bioactivos presentes en la corteza y semillas de las variedades dulce y amargas del fruto del rambután y su capacidad antioxidante.

Métodos: Se realizó a través de un análisis farmacognóstico de la corteza de los frutos, basado en la evaluación de los parámetros fisicoquímicos, el análisis fitoquímico, la cuantificación de los fenoles y flavonoides, la determinación de la capacidad antioxidante y la identificación de los compuestos grasos presentes en las semillas mediante cromatografía de gases acoplada a espectrometría de masas.

Resultados: Los resultados mostraron que tanto las cortezas como las semillas de las dos variedades difieren en el contenido de fitoconstituyentes y sus propiedades antioxidantes.

Conclusiones: Los frutos amargos presentaron una mayor concentración de fenoles y flavonoides en la corteza y una mayor concentración de compuestos grasos en las semillas, con marcadas diferencias entre las fracciones de los compuestos no saponificables. Para la fracción de ácidos grasos, se evidenció una mayor similitud cualitativa, con diferencias cuantitativas. Aunque los extractos de la corteza de ambos frutos tienen capacidad antioxidante, fue significativamente mayor en los frutos amargos.

Palabras Clave: antioxidante; compuestos fenólicos; compuestos grasos; corteza de fruto; semillas.

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INTRODUCTION

In Ecuador there is a wide variety of plant species that are important for their pharmacological and nutritional properties, however, most do not have scientific studies regarding their chemical composition and therapeutic benefit (Van Baren, 2015).

Among the biodiversity, rambutan is highlighted, a fruit native to the region of Malaysia that was incorporated to the Ecuadorian agricultural sector in 1960 as an ornamental bush (Arias and Calvo, 2014). Its oval shaped fruit presents a red or yellow pericarp covered in its totality by soft white thorns that vary in their tonality (green, yellow, red). It consists of a translucent, sweet and juicy edible aryl, the seed is characterized by its light brown coloration and high fat content (Hernández et al., 2011; Do Sacramento and Aparecida., 2014).

Traditionally it is considered a health food capable of providing a high content of vitamins, proteins, carbohydrates and minerals, however, 60% of metabolites with therapeutic properties found in the seeds have high amounts of fatty acids, including those of higher relevance: oleic and arachidonic acid that reach up to 53.76% in its composition (García, et al., 2016; Hernandez, et al., 2016).

One of the most important elements in *N. lappaceum* is its seed; the proximal percentage of basic compounds found in the seed are: Fiber (2.8-11.6%), carbohydrates (28.7-48.1%), protein (7.8-14.1%) and fat (33.4-38.9%) (Mahisanunt et al., 2017).

Within the medicinal properties of the fruit, it was shown by Rahayu et al. (2013), that the infusion of mature seeds at concentrations of 3.12 g/kg possess reconstructive activity of beta-pancreatic cells and that oral administration of the extract decreases considerably the levels of hyperglycemia in blood. The antioxidant capacity of fat of the extracted seed has also been evaluated by means of isothermal fractionation techniques with reference solvents (acetone and ethanol) (Mahisanunt et al., 2017).

Regarding its toxicity Eiamwat et al. (2014), conducted *in vivo* studies in rats and rabbits from acute oral, dermal and irritant tests, of the oils extracted from the seeds and determined that they do not have significant toxic effects and that they are safe for use in industrial applications both in food and cosmetics.

Okonogi et al. (2007) reported that the fruit's peels had antioxidant capacity against 2,2-diphenyl-1-picril hydracil (DPPH), 2,2'-azinobis (3-ethylbenzothiazolin) -6-sulfonic acid (ABTS) and antioxidant capacity equivalent to Trolox (TEAC), also had no toxic activity against heterogeneous human epithelial colorectal adenocarcinoma cells (Caco 2).

Thitilertdecha (2010), determined in different extracts of the peels of the fruit of the species (ethereal, methanolic and aqueous), the concentration of polyphenols, antioxidant and antimicrobial activity; finding that the methanolic extract had the highest content of phenolic compounds and the highest antioxidant activity and that all the extracts had antimicrobial activity against pathogenic bacteria.

The ethanol extract of the peels of the fruit was tested in diabetic rats induced with aloxane, finding a decrease in blood sugar levels, which suggests an anti-diabetic activity (Muhtadi et al., 2015).

On the other hand, Wanlapa et al. (2016) and Rodrigues et al. (2018) consider that the use of waste, constitute a field of research in the search for active metabolites in order to give an added value to them. Given this background, the objective of this work was to identify the bioactive metabolites present in the peels and seeds of the sweet and bitter varieties of the fruit and the antioxidant activity of the peels, since it constitutes waste from the food industry.

MATERIAL AND METHODS

Harvesting, drying and grinding

The plant material under study were the fruits and were collected between the months of June and August 2018 at the Farm of Mr. Roca Coque situated in the Province of Los Ríos canton Quevedo, Ecuador, located at 1°1'49"S and 79°24´48"E. Adult plants of approximately 15 to 25 m high were chosen in the flowering-fruiting state. For the experimental analyzes of the investigation, two varieties of fruits, sweet and bitter, were used, differentiated by their color and size (Fig. 1).

Figure 1. Fruit peels of *Nephelium lappaceum* L. **(A)** sweet variety, **(B)** bitter variety.

The taxonomic characterization was performed in the herbarium of the Faculty of Natural Sciences of the University of Guayaquil, where the voucher GUAY-13.114 was given. The fruits were peeled separately and the peels washed with plenty of water to remove all the pulp, then washed with distilled water and dried in an air recirculation oven at 50°C to constant weight, after which they were milled in mill of blade until particle size of 2 mm and were stored in glass jar for later analysis.

The seeds were extracted from the fruit, washed to remove pericarp waste, dried in an air recirculation oven at 50°C and milled to a particle size of 2 mm.

Physicochemical parameters and phytochemical screening

The physicochemical parameters and the qualitative composition were determined to the dust of the peels and the seeds of the two varieties through phytochemical screening, for which the procedures described by the WHO (2011) and Miranda and Cuéllar (2012) were followed. For sequential extraction 10 g of sample, 50 mL of diethyl ether, ethanol and distilled water were used. The reagents used were pure for analysis from Merck, Germany.

Obtaining the hydroalcoholic extract of the peels

From the powder of the peels of the two varieties, extracts were made at a rate of 20 g of drug per 100 mL of solvent, by the method of maceration, for a period of seven days, with stirring in shaker, at a temperature of 30°C ± 2°C, using an 80% hydroalcoholic mixture as solvent.

Quantification of phenols and total flavonoids

The hydroalcoholic extract of the fruit crusts under study was used. The total phenolic content was determined by the Folin-Ciocalteu method (Pourmorad et al., 2006; Memnune et al., 2009; Chlopicka, 2012). Merck gallic acid was used as the reference substance. Quantification of total flavonoids was carried out by the colorimetric method of aluminum trichloride (Chang et al., 2002; Pourmorad et al., 2006) and the quercetin standard Merck, was used as reference substance.

Fat extraction and physicochemical properties

The oils of the seeds (20 g) were extracted with 150 mL hexane Merck by the Soxhlet method for a period of 3 hours, after which the solvent was removed, their yields were calculated and quality parameters such as refractive index, relative density, saponification index and esterification, according to the Ecuadorian Technical Standards NTE INEN (35; 40; and 42, 1973) were measured.

Saponification and methylation of fats

To 1 g of the fatty compounds 20 mL of 20% sodium hydroxide in ethanol were added and refluxed for two hours, the fractions of fatty acids and unsaponifiable compounds were separated according to the procedure described by Miranda and Cuellar (2012); fatty acids (100 mg) were converted to methyl ester by treatment with 2 mL of hexane and 1 mL of sodium hydroxide 2 M in methanol. The reaction was stirred, it was heated at 70°C for six minutes, and it was centrifuged to 5000 rpm for 3 minutes. All reagents used were from Merck.

Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS analysis was performed in a gas chromatography mass spectrometry equipment Agilent Technologies (7890A GC system and 5975C inert XL MSD with triple axis detector). A capillary column DB-5MS (30 m × 0.25 mm) with phenyl dimethylpolysiloxane was used as stationary phase (0.25-micron film thickness) and helium as the carrier gas. The injection of 1 µL of saponifiable fraction was done with split mode (50:1) and a flow of 1.0 mL/min. The oven temperature was maintained at 150° C for 4 minutes, and then it was increased to 250°C at 4°C/min.

On the other hand, the injection of 1 µL of non-saponifiable fraction was done with splitless mode and a flow of 1.2 mL/min. The temperature of the injection system was 250°C for both samples. The oven temperature was started at 70°C for 2 minutes, then it was increased to 300°C at 5°C/min, and it was maintained at 300°C for 6 minutes. The compounds identification was done by comparison of mass spectra based on Wiley 9th with NIST 2011 MS Library. An electron ionization of 70 eV at 230°C was used in the ion source and the data compounds were collect with the full scan mode (40 - 600 amu) in the quadrupole mass analyzer.

Biological activity

Determination of antioxidant capacity

The reagents used in these determinations were from Sigma Aldrich.

Antioxidant activity by the FRAP method (ferro-reducing capacity)

The capacity to reduce hydroalcoholic extracts was measured according to the procedure described by Benzie and Strain (1996). The determinations were spectrophotometric, a UV-visible spectrophotometer was used at an absorbance of 593 nm.

The samples tested were hydroalcoholic extracts at 80% peels of *Nephelium lappaceum*, sweet and bitter variety at the concentrations of 2, 10, 20, 30 and 40 µg/mL.

The results were expressed as µmol equivalents of ascorbic acid (EAA) and as µmol equivalents of FeSO4, from the calculation interpolating the optical density (OD) of the samples in the calibration curves of both reference substances at concentrations of 100, 400, 800, 1000 and 1500 µM. The readings were made in triplicate at four minutes intervals.

Scavenging capacity of the 2,2-diphenyl-1-pyrylhydrazyl radical (DPPH)

For the quantitative determination, the DPPH free radical method (2,2-diphenyl-1-picrylhydracil radical) was used. A UV-visible spectrophotometer was used, and the determinations were measured at 517 nm after 30 min. (Brand-Williams et al., 1995; Kedare and Singh, 2011). Hydroalcoholic extracts were used at 80% of the peels of *Nephelium lappaceum*, sweet and bitter variety concentrations of 5; 12.5; 25; 37.5 and 50 µg/mL. The results were

expressed as a percentage of DPPH radical inhibition according to the following formula [1]:

$$\% \text{ DPPH radical inhibition} = [(Ab - Am)/Ab] \times 100 \quad [1]$$

The mean inhibitory concentration (IC_{50}) was determined with the help of the statistical program Graphprism 5.0. Values close to 20 µg/mL were considered of interest.

ABTS●+ test (2,2′-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid)

The trial was performed according to the methodology of Re et al. (1999), Arnao et al. (2001) and Agudo (2010). The results were expressed as percent inhibition of the ABTS●+ radical according to the following formula [2]:

$$\% \text{ inhibition} = [A734 (ABTS) - A734 (antioxidant)]/ A734 (ABTS) \times 100 \quad [2]$$

Hydroalcoholic extracts were used at 80% of the bark of *Nephelium lappaceum*, sweet and bitter variety concentrations of 500, 600, 700, 800 and 900 µg/mL.

Statistical analysis

Physicochemical parameters were determined in triplicate and expressed as mean value ± standard deviation. The differences were evaluated by analysis of variance (ANOVA 1) and the comparison of means by the Turkey test for p>5%.

For the processing and statistical analysis of the antioxidant study, the statistical program SPSS for Windows version 8.0 was used. The experimental values were expressed as the mean ± standard deviation (SD). Data from the DPPH and ABTS trials were analyzed by ANOVA 1, followed by a test of multiple comparisons of Tukey media with a p≤0.05. The mean values of the two extracts in the FRAP trial were analyzed by student's t.

RESULTS AND DISCUSSION

Physicochemical parameters

Physicochemical parameters determined to the dried and ground plant are presented in Table 1.

The residual humidity after drying has a low value, which guarantees adequate conservation once the seeds have been crushed. It should be noted that the seeds of the sweet variety have a higher percentage of moisture, presumably due to the greater amount of sugar found in them in comparison to the bitter seeds. The residual moisture values for the peels of the fruits are higher than that of the seeds, but are within the range reported in the literature for plant drugs, although the humidity values for the peels of the bitter variety are lower than that of the Sweet variety, do not differ significantly, contrary to what happens for the seeds.

Table 1. Physicochemical parameters of the seeds and peels of the fruits of *Nephelium lappaceum* L.

Parameters		Sweet variety seeds	Bitter variety seeds	Sweet variety peels	Bitter variety peels
Residual humidity		3.997 ± 0.005^{a}	2.640 ± 0.01^{b}	10.00 ± 1.41^{a}	9.5 ± 0.70^{a}
Total Ashes		1.469 ± 0.002^{c}	1.498 ± 0.001^{c}	2.93 ± 0.05^{n}	2.83 ± 0.04^{o}
Insoluble ash in hydrochloric acid		1.240 ± 0.001^{d}	1.34 ± 0.001^{e}	1.75 ± 0.31^{r}	1.68 ± 0.17^{r}
Soluble substances	Hexane	14.680 ± 0.000^{f}	3.960 ± 0.000^{g}	0.38 ± 0.11^{m}	0.30 ± 0.04^{m}
	Ethanol	3.414 ± 0.000^{h}	3.528 ± 0.000^{h}	10.46 ± 0.86^{j}	12.96 ± 0.27^{k}
	Ethyl acetate	8.660 ± 0.000^{i}	7.513 ± 0.000^{j}	2.53 ± 0.23^{l}	2.87 ± 0.15^{l}

Data are expressed as percentage ± standard deviation of n = 5. Different letters indicate significant differences p<0.05 between the variables for the same parameter.

The total ashes reflect the presence of minerals present in the plant, which are absorbed from the soil; various pharmacopoeias indicate up to 5% of ash percentages (WHO, 2011), depending on the vegetable organ studied. Total ash values are within the allowable ranges for plant drug and both types of seeds did not differ significantly. For fruit crusts, the values are higher, but do not exceed the established value. In this case, significant differences were observed between the varieties studied. The bitter variety presented less value.

For the insoluble ashes, which fundamentally reflect the presence of heavy metals, the Pharmacopoeias raise values no higher than 2%. For the studied species the percentage of ashes insoluble in hydrochloric acid is very important because, despite being within the permissible values, they are very close to the values obtained for the total ashes, resulting in differences of significance between the two varieties of seeds, not so of the peels. However, it is important to highlight the results obtained by Wall (2006) who points out that the fruits of the species are rich in mineral salts and report the presence of macro-elements such as phosphorus, potassium, calcium, magnesium and sodium and within the microelements the presence of iron, manganese, zinc and copper; logically concentration and presence of these depends on the type of soil where the species grows.

The determination of soluble substances is one of the parameters that provides the most information when selecting the best solvents in the extraction process. These were evaluated in three types of solvents (hexane, ethanol and ethyl acetate) according to the hot method recommended by the WHO (2011). Hexane was shown to be the most suitable solvent for the extraction of soluble substances in sweet seeds, not being a good solvent for bitter seeds and for the peels of both varieties. Ethyl acetate could extract the components of the seeds very well, but not from the peels and ethanol, it was the solvent that gave the lowest values for both seeds and higher for the peels.

In this test, significant differences were found for the seeds of the two varieties with the hexane and ethanol solvents, while for the peels these were only revealed with the solvent ethanol.

It is worth mentioning the differences found in the percentage of hexane soluble substances between sweet and bitter seeds, which may be indicative of a marked difference in the concentration of low polarity substances.

Qualitative chemical study

The presence of secondary metabolites was similar in both seeds and peels, with some differences in the intensity of the coloring reactions indicative of differences in concentration. The main metabolites detected were: Fatty compounds, triterpenes and/or steroids, reducing compounds, saponins, phenols and tannins, quinones, flavonoids, resins and bitter compounds. With the exception of phenols and tannins and flavonoids, the rest of the compounds showed more intense reactions in the seeds.

The presence of fatty acids is common for the seeds of the species and their composition has been reported. For the phenolic compounds its presence in the fruit has been reported by Thitilertdecha and Rakariyatham (2011), who inform different phenolic compounds as components of the fruits such as geraniine which is an ellagitannin, corilagina and ellagic acid; the presence of these and other phenolic compounds tannin type, was ratified by Perera et al. (2012), while Prakash et al. (2017), came to separate by ultrasonic techniques the anthocyanidins, flavonoids and phenolic compounds present in the fruits. Triterpenoids are usually part of the unsaponifiable fraction of fats, although no reports of their presence have been found.

Quantification of phenols and total flavonoids

Taking into account the results obtained in the qualitative analysis of the fruit crusts and the information collected from the literature, the concentration of total phenols and total flavonoids was evaluated, and the results are presented in Table 2.

Table 2. Total phenol content and total flavonoids in peels extracts of two varieties of *N. lappaceum* L.

Extracts	Total flavonoids mg/mL	Total phenols mg/mL
Sweet variety	3.60 ± 0.11[a]	22.42 ± 0.26[a]
Bitter variety	4.07 ± 0.18[b]	23.98 ± 0.47[b]

Data are expressed as mean ± standard deviation (n=3). Different letters show statistically significant differences ($p<0.05$) for 95% confidence, according to t-Student.

Table 3. Physicochemical parameters of the oil of the seeds of the two varieties of the fruit of *Nephelium lappaceum*.

Parameters	Sweet variety seeds	Bitter variety seeds
Performance	57.350 ± 0.080[a]	68.888 ± 0.030[b]
Density	0.746 ± 0.001[c]	0.815 ± 0.001[d]
Refractive index	1.436 ± 0.001[e]	1.459 ± 0.001[f]
Saponification Index	191.28 ± 0.040[g]	215.05 ± 0.010[h]
Esterification index	146.39 ± 0.050[i]	185.37 ±0.020[j]

Data are expressed as percentage ± standard deviation of n = 5. Different letters indicate significant differences $p<0.05$ between the variables for the same parameter.

In both varieties, phenols and flavonoids were found, nevertheless the flavonoid concentration was higher. The statistical analysis of the results showed significant differences in the content of these metabolites in the two extracts evaluated. The concentrations of total phenols and total flavonoids being higher for the hydroalcoholic extract from the bitter variety.

Extraction and analysis of fatty compounds

The process enabled the confirmation of a high fat yield in the seeds of the fruits of the two varieties of the species; it was found that the seeds of the bitter variety had a higher percentage of fatty compounds than those of the sweet variety, and that all parameters evaluated as density, refractive index, saponification index and esterification index had higher values for these seeds (Table 3).

The gaseous chromatograms of the unsaponifiable fractions of the seeds of the fruits of the two varieties are presented in Fig. 2A-B.

The compounds identified for these fractions are described in Table 4.

Solís Fuentes et al. (2010), reported for the oil of rambutan seeds, without specifying the variety, values of refractive index of 1.468 ± 0.001 similar to that obtained for the seeds of the bitter variety; the reported saponification index was 1.86, in this case similar to that of the seeds of the sweet variety.

Once the oil was saponified and the unsaponifiable fractions and the fatty acids separated, the corresponding analysis was performed by gas chromatography coupled to mass spectrometry.

Differences were observed in the chromatographic profiles of the fractions of unsaponifiable compounds of the seeds of the bitter and sweet varieties. The team recorded a total of 156 chromatographic peaks for the extract of the seeds of the bitter variety and 185 for that of the sweet variety, although not all could be identified by comparison of their mass spectra with those of the team's library.

Of the 156 peaks recorded in the chromatogram of the fruits of the bitter variety, 6 and of the 185 recorded in the fruits of the sweet variety 13 could be identified. Three coincidences were found among the unsaponifiable compounds identified in the seeds of both fruits, although with difference of abundances between them.

Table 4. Compounds of non-saponifiable fraction and saponifiable fraction of sweet and bitter *N. lappaceum* seeds.

COMPOUNDS OF NON-SAPONIFIABLE FRACTION				
PEAK	**Compound**	**Molecular formula**	**Relative abundance (%)***	
			Sweet seeds	**Bitter seeds**
1	Octadecane	$C_{18}H_{38}$	0.11 ± 0.00	ND
2	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	0.33 ± 0.01	ND
3	9-Hexadecenoic acid ethyl ester	$C_{18}H_{34}O_2$	6.53 ± 0.34	ND
4	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	ND	37.00 ± 0.42
5	9-Octadecenoic acid	$C_{18}H_{34}O_2$	ND	5.25 ± 0.07
6	9,12 Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	0.44 ± 0.01	ND
7	9-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	0.78 ± 0.01	ND
8	Octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	0.33 ± 0.00	ND
9	Erucic acid ethyl ester	$C_{24}H_{46}O_2$	10.30 ± 0.03	ND
10	n-Hexacosane	$C_{26}H_{54}$	3.99 ± 0.02	ND
11	Eicosane	$C_{20}H_{42}$	16.61 ± 0.33	4.39 ± 0.15
12	Octacosane	$C_{28}H_{58}$	ND	7.60 ± 0.01
13	Tetracosane	$C_{24}H_{50}$	6.09 ± 0.10	ND
14	Squalene	$C_{30}H_{50}$	5.87 ± 0.04	4.39 ± 0.08
15	Stigmasterol	$C_{29}H_{40}O$	1.66 ± 0.03	12.11 ± 0.13
COMPOUNDS OF SAPONIFIABLE FRACTION				
PEAK	**Compound**	**Molecular formula**	**Relative abundance (%)***	
			Sweet seeds	**Bitter seeds**
1	Hexadecanoic acid	$C_{16}H_{32}O_2$	4.27± 0.03	4.47 ± 0.30
2	9,12 Octadienoic acid	$C_{18}H_{32}O_2$	1.47 ± 0.02	1.56 ± 0.13
3	9-Octadecenoic acid	$C_{18}H_{34}O_2$	42.25 ± 0.26	38.4 ± 0.30
4	Octadecanoic acid	$C_{18}H_{36}O_2$	6.38 ± 0.13	5.89 ± 0.08
5	Eicosanoic acid	$C_{20}H_{40}O_2$	35.85 ± 0.21	31.96 ± 0.84
6	Docosanoic acid	$C_{22}H_{44}O_2$	1.57 ± 0.18	12.15 ± 1.29

*Data are expressed as mean values ± standard deviation (n=3), nd: not detected.

The fractions were mainly constituted by esters of fatty acids and hydrocarbons. The major components of the seeds of the bitter variety fruits were methyl palmitate (37.00%) and stigmasterol which presented a percentage of 12.11%, the rest of the components did not reach abundances higher than 10%. For the seeds of the sweet variety the major components were eicosane (16.61%).

For the fraction of unsaponifiable compounds of the fruits, no information is recorded in the consulted bibliography.

For the fraction of methylated saponifiable compounds, analytical gaseous chromatograms are presented in Fig. 2C-D.

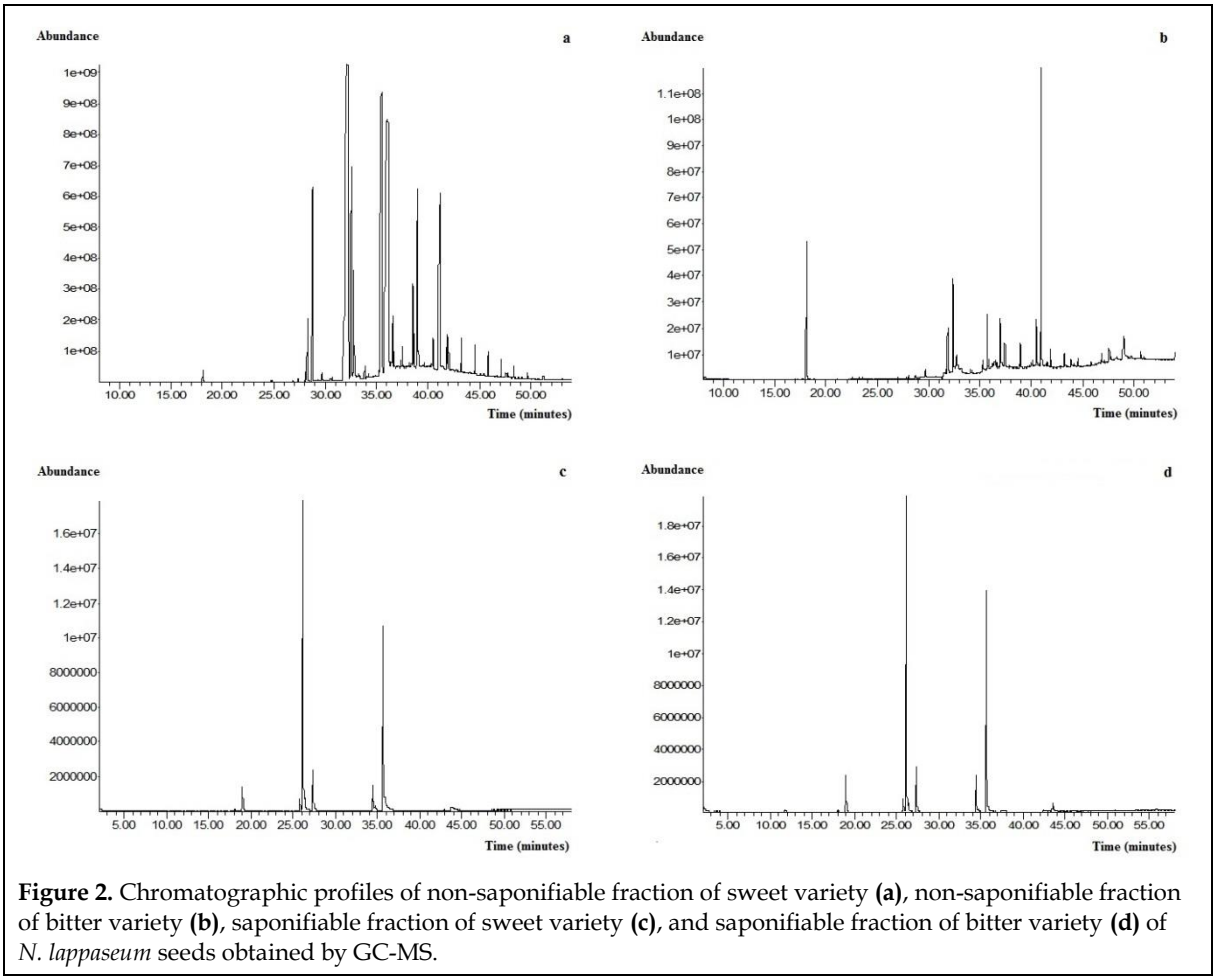

Figure 2. Chromatographic profiles of non-saponifiable fraction of sweet variety **(a)**, non-saponifiable fraction of bitter variety **(b)**, saponifiable fraction of sweet variety **(c)**, and saponifiable fraction of bitter variety **(d)** of *N. lappaseum* seeds obtained by GC-MS.

For these compounds a greater coincidence was observed, in the chromatograms, with differences in the intensities of the chromatographic peaks. For the fraction of saponifiable compounds of the seeds of the bitter variety fruits, the team registered a total of 11 chromatographic peaks, while for the sweet variety 9 were recorded. Table 4 lists the compounds identified for the fractions of both varieties.

In both fractions six fatty acids could be identified and the differences between the two varieties were quantitative. Solís Fuentes et al. (2010), pointed out that in the oil of rambutan seeds the main fatty acids were 40.3% oleic acid; 34.5% arachidic acid; 6.1% palmitic acid; 7.1% stearic acid and 2.9% behenic acid. According to Mariod et al. (2017), the oil was mainly constituted by the saturated fatty acids: palmitic (7.39 - 10.33%), stearic (12.21 - 16.58%), arachidic (12.34 - 16.22%) and behenic (6.53 - 8.91%) and oleic (50.17 - 52.18%) as monounsaturated fatty acid and linolenic acid (2.02% - 3. 04%) as a polyunsaturated fatty acid. On the other hand, Mahisanunt et al. (2017), pointed out that the largest fatty acid in the fraction was arachidic acid with more than 90%.

Although different varieties have been reported for rambutan, none of the scientific papers consulted specify the variety that has been studied. However, it is important to point out that the fatty acids reported in the literature are all present and their quantitative variations may be due to the ecological-geographical conditions where the species develops.

Determination of antioxidant capacity

Antioxidant activity by the FRAP method (ferro-reducing capacity)

Table 5 shows the ferro-reducing activity associated with the two extracts evaluated. A concentration-dependent antioxidant activity was evidenced, achieving at all the concentrations tested for both extracts, higher values (in equivalents of ascorbic acid and $FeSO_4$) at the lowest concentration tested (100 µM) of each reference substance.

The statistical analysis showed that there were significant differences between the equivalent µM values of ascorbic acid for extracts of the sweet and bitter variety at all the concentrations tested. The behavior was similar for the equivalent µM of $FeSO_4$. The greatest capacity for reducing Fe^{3+} was manifested at the highest evaluated concentrations, being higher for the bitter variety.

The results allowed us to suggest that the two extracts had a high antioxidant activity, which translates into high equivalent µM values, expressed as a function of the reference substances tested.

Sequestration capacity of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH)

As can be seen in Table 5, in general terms there were significant differences between the samples at the same concentration, except for the extract of the sweet variety that presented a similar behavior with the Trolox pattern at the concentrations of 37.5 and 50 µg/mL, and higher than vitamin C. On the other hand, the extract of the bitter variety showed the highest percentage of DPPH radical inhibition at all the concentrations evaluated, with significant differences with respect to the two reference substances.

The notorious element of the study is that the extracts of sweet and bitter peels showed percentages of inhibition greater than 50% at very low concentrations, which speaks in favor of a high antioxidant effect by the evaluated mechanism of action.

Of the samples evaluated, the one with the lowest IC_{50} (concentration value at which 50% inhibition of the maximum DPPH sequestration effect is reached) was the extract obtained from the bitter variety with a value of 9.72 µg/mL, followed by extract of the sweet variety, indicating better antioxidant effect with respect to the reference substances.

According to this technique, samples that at a concentration close to 20 µg/mL inhibit 50% of DPPH discoloration are considered of interest, so it can be considered that under these working conditions, the extracts have a good antioxidant power.

ABTS●+ test (2,2'-azino-bis (3-ethylbenzothiazoline) - 6-sulfonic acid)

Table 5 shows the percentages of inhibition of the ABTS radical. Reference substances achieved the highest percentages of radical inhibition, from the lowest concentration tested (500 µg/mL). However, the two extracts evaluated also showed a high antioxidant activity, with inhibition percentages greater than 70% from 300 µg/mL, the higher being for the extract from the bitter variety.

The statistical analysis of the results allowed to detect significant differences between the extracts at 500 and 600 µg/mL, but not at the concentrations of 700 to 900 µg/mL; highlighting the extract of the bitter variety with a behavior similar to vitamin C and Trolox at the highest concentration. On the other hand, the two reference substances did not show significant differences in the percentages of radical inhibition in the five concentrations tested.

Of the samples evaluated, those that presented lower IC_{50} (concentration value at which 50% inhibition of the maximum abduction effect of ABTS is reached) were Trolox and the extract obtained from the bitter variety with values of 647.50 and 651.70 µg/mL, respectively, indicating a better antioxidant effect than the extract of the sweet variety.

Oxidative stress contributes significantly to the pathogenesis of some chronic diseases, therefore, that antioxidant behavior is one of the most com-

monly determined biological activities in plant extracts. There is a wide variety of tests to determine the antioxidant activity of plant extracts, two of them are based on the elimination of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) (DPPH test) and the potential for reduction activity iron (FRAP test).

Some studies use the DPPH and FRAP assays for the detection of plant antioxidant activity, assuming that a combination of the data would provide a better description of the antioxidant activity than that obtained from a single test (Clarke et al., 2013).

Table 5. Antioxidant activity of peels extracts of sweet and bitter varieties of *Nephelium lappaceum* L.

Ferro-reducing activity of *Nephelium lappaceum* extracts				
Concentration µg/mL	**Ferro-reducing capacity ± SD**			
	µM equivalent of ascorbic acid		**µM equivalent of $FeSO_4$**	
	Sweet variety	**Bitter variety**	**Sweet variety**	**Bitter variety**
2	180.85 ± 11.18^{a}	367.52 ± 29.00^{b}	107.05 ± 11.44^{k}	297.96 ± 29.66^{l}
10	357.15 ± 16.77^{c}	632.71 ± 25.62^{d}	287.36 ± 17.15^{m}	569.17 ± 26.21^{n}
20	811.22 ± 25.27^{e}	966.04 ± 15.56^{f}	759.33 ± 18.92^{o}	910 09 ± 15.91^{p}
30	891.96 ± 17.82^{g}	1261.60 ± 52.06^{h}	834.32 ± 18.23^{q}	1212.36 ± 53.24^{r}
40	1305.30 ± 19.15^{i}	1452.71 ± 33.18^{j}	1257.06 ± 1959^{s}	1407.81± 33.93^{t}
DPPH scavenging capacity of *Nephelium lappaceum* extracts and reference substances				
Concentration µg/mL	**DPPH radical sequestration percentage (%) ± SD**			
	Sweet variety extract	**Bitter variety extract**	**Vitamin C**	**Trolox**
5	52.27 ± 0.80^{a}	60.11 ± 0.78^{b}	56.08 ± 1.48^{c}	64.66 ± 1.18^{d}
12.5	76.06 ± 0.66^{e}	87.08 ± 0.68^{f}	70.47 ± 0.72^{g}	72.25 ± 1.16^{h}
25	86.93 ± 0.83^{i}	88.89 ± 1.01^{j}	78.42 ± 0.13^{k}	84.34 ± 0.90^{l}
37.5	88.49 ± 0.67^{m}	90.08 ± 0.34^{n}	85.45 ± 1.12^{o}	87.23 ±0.72^{om}
50	89.49 ± 0.55^{p}	90.82 ± 0.51^{q}	87.86 ± 1.00^{r}	88.86 ± 0.61^{rp}
IC_{50} (µg/mL)	11.05 ± 3.18^{s}	9.72 ± 2.58^{t}	14.84 ± 6.87^{u}	15.94 ± 3.78^{v}
ABTS●+ radical scavenging capacity of *Nephelium lappaceum* extracts and the reference substance				
Concentration µg/mL	**Scavenging percentage of radical ABTS●+ (%) ± SD**			
	Sweet variety extract	**Bitter variety extract**	**Vitamin C**	**Trolox**
500	33.78 ± 2.16^{a}	41.43 ± 2.04^{b}	90.15 ± 0.78^{c}	89.78 ± 1.50^{c}
600	38.32 ± 1.48^{d}	61.99 ± 1.60^{e}	92.57 ± 0.76^{f}	91.80 ± 1.24^{f}
700	70.51 ± 1.78^{g}	73.34 ± 2.44^{g}	92.90 ± 1.72^{h}	93.81 ± 1.19^{h}
800	88.13 ± 1.69^{i}	87.54 ± 1.60^{i}	94.36 ± 1.17^{j}	95.18 ± 0.68^{j}
900	93.03 ± 1.17^{k}	94.22 ± 1.80^{kl}	96.42 ± 0.73^{l}	95.73 ± 1.39^{l}
IC_{50} (µg/mL)	682.80 ± 2.96^{m}	651.70 ± 6.99^{n}	693.70±18.86^{o}	647.50 ± 18.63^{p}

Data are expressed as mean ± standard deviation (SD) (n = 3). Different letters in the same row indicate significant differences ($p \leq 0.05$), according to the Tukey multiple comparison test.

The ABTS and DPPH methods correlate with each other since they are based on one of the most applied strategies in the *in vitro* measurement of the total antioxidant capacity of a compound, which consists in determining the activity of the antioxidant against chromogenic substances of a radical nature, so that the change in color intensity occurs proportionally to the concentration of antioxidants in the sample (Kuskoski et al., 2005).

On the other hand, the presence of different antioxidants in a mixture or extract causes synergism or antagonism and makes the antioxidant activity not only depend on its concentration but also on the interaction amongst them. The antioxidant capacity of an extract is not only given by the sum of the antioxidant capacities of each of its components, it also depends on the microenvironment in which they are found and the interactions that occur between them (Kuskoski et al., 2005).

CONCLUSIONS

The species under study was naturalized in Ecuador and no scientific studies had been conducted for any of the introduced varieties.

Differences were found in the composition and properties of the peels and seeds of the two varieties studied.

The bitter variety showed a higher concentration in bioactive metabolites than the sweet variety and a superior antioxidant capacity.

Although different varieties of this species are reported in the literature, the variety studied is not established in any of the published scientific papers, it being demonstrated in this work that there may be significant differences between them.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION:

Contribution	Valdez LL	Chóez IA	Carrillo GA	Miranda M
Concepts or ideas	x	x	x	x
Design	x	x		x
Definition of intellectual content	x	x	x	x
Literature search	x			
Experimental studies	x	x		
Data acquisition	x	x		
Data analysis	x	x		x
Statistical analysis	x	x		x
Manuscript preparation	x		x	x
Manuscript editing		x	x	x
Manuscript review	x	x	x	x

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