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## Polyphenolic content, *in vitro* antioxidant activity and chemical composition of extract from *Nephelium lappaceum* L. (Mexican rambutan) husk

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## ABSTRACT

**Objective:** To determinate the recovery of total polyphenolic compounds content, *in vitro* antioxidant activity and HPLC/ESI/MS characterization of extract from *Nephelium lappaceum* L. (Mexican rambutan).

**Methods:** The rambutan husk extract was obtained by aqueous extraction and a polyphenolic fraction was recovered using Amberlite XAD-16. The total polyphenolic compounds content was determined by the Folin Ciocalteu and butanol-HCl methods. *In vitro* antioxidant activity was performed using ABTS and ferric reducing antioxidant power methods.

**Results:** Mexican rambutan husk showed a total polyphenolic content of 582 mg/g and an evident antioxidant activity by ABTS and ferric reducing antioxidant power analysis. The HPLC/ESI/MS assay allowed the identification of 13 compounds, most of which belong to ellagitannins. Geraniin, corilagin and ellagic acid were present in the sample; the mineral composition was also evaluated.

**Conclusions:** Rambutan husk cultivated in Mexico is a promising source for the recovery of added value bioactive compounds with antioxidant activity, which have potential applications as bioactive antioxidant agents for the treatment of diseases.

## 1. Introduction

*Nephelium lappaceum* L. (rambutan) is a tropical fruit native from southeast Asia (mainly Malaysia, Indonesia and Thailand) that belongs to the Sapindaceae family. It is an ovoid red or yellow fruit which is sweet, juicy with rich vitamin C [1]. The cultivation of this fruit in Mexico began in 1950 [2], mainly in Chiapas state [3], although its cultivation has spread to other parts of Mexico, such as Veracruz, Tabasco and Nayarit states [4]. Today in Mexico, the rambutan culture is commercial and its consumption is in fresh or processed products [3]; however, wastes generated after industrialization, such as husks which

have been not exploited yet. Interest in the consumption and utilization of this fruit has increased in recent years, since it has been shown that the fruit contains polyphenolic bioactive compounds [5,6], antimicrobial activity [7,8] and antihyperglycemic activity [9,10], which are considered natural compounds with high added value and with high antioxidant activity. Polyphenolic compounds such as geraniin, corilagin and ellagic acid with high antioxidant activity have been identified as the main compounds in rambutan [6]. In addition, other polyphenolic compounds have been reported with important biological activities as anticarcinogenic agents such as benzo [a] pyrene-7,8-diol-9,10-epoxide [11], which generates mutations in healthy human cells [12]. These compounds can play a role against parasites such as *Leishmania donovani* and also act as antiviral agents which can inhibit the replication of human immunodeficiency virus (HIV) [13] and prevent the disease known as leishmaniasis [14]. According to the literature reviewed, there are no studies to identify phenolic compounds

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from rambutan (husks) cultivated in Mexico; in this sense, it is important to generate information related in this field because this fruit represents a potential source for the recovery of polyphenolic compounds with high added value. Therefore, the aim of this study is the separation and identification of polyphenolic compounds from rambutan (husk) cultivated in Mexico by liquid chromatography and mass spectrometry.

## 2. Materials and methods

### 2.1. Plant material

Rambutan was obtained from the Soconusco region, Comitán, Chiapas, Mexico. Fruit husks were removed manually and dehydrated at 60 °C by 48 h in an oven; these conditions have been reported as safe for polyphenolic compounds [15,16], then the dehydrated material was ground in a blade mill.

### 2.2. Extraction and purification of rambutan husk polyphenols

The rambutan husk polyphenols were extracted from dried plant material (20 g) with distilled water (100 mL) at 60 °C (m/v ratio 1:5). The mixture was placed in a heating oven at 60 °C to maintain the extraction temperature by 30 min. After extraction, the mixture was filtered through Whatman No. 41 membranes [15]. Polyphenols fraction was obtained by using a liquid chromatography column with Amberlite XAD-16, first elution was made with distilled water, then ethanol was used to recover the phenolic fraction [16].

### 2.3. Determination of total hydrolysable polyphenols

The total hydrolysable (poly)phenols from rambutan husk extract were determined by using Folin's Ciocalteu reagent [15]. The experiment was performed by triplicate, the content of total hydrolysable (poly)phenols was expressed as gallic acid equivalents (GAE).

### 2.4. Determination of total condensed polyphenols

The total condensed polyphenols from rambutan husk extract were determined by using ferric reagent and butanol-HCl [17]. The experiment was performed by triplicate, the content of total condensed polyphenols was expressed as catechin equivalents.

### 2.5. ABTS antioxidant assay

ABTS-scavenging capacity assay was carried out according to the methodology proposed by Re *et al.* [18]. A stock solution containing 7 mM ABTS solution and 2.45 mM of potassium persulfate (2:1) was rested from 12 h to 16 h at room temperature and adjusted with absolute ethanol until reaching an absorbance of  $(0.700 \pm 0.002)$  nm according to the original methodology. After that, the measuring cell was placed in the spectrophotometer and 50  $\mu$ L of sample and 950  $\mu$ L of the above resulting solution were added. After 1 min, the absorbance was measured at 734 nm. The results were expressed as half maximal inhibitory concentration according to the calibration curve prepared by using purified polyphenolic compounds from rambutan husk.

### 2.6. Ferric reducing antioxidant power

The method of Gow-Chin and Hui-Yin [19] was adopted to determine the reduction power in the samples with slight modifications. Briefly, 50  $\mu$ L of the sample at 500 mg/mL was mixed with 120  $\mu$ L of phosphate buffer (0.1M, pH 6.6). Then, 120  $\mu$ L of potassium ferricyanide were added to the mixture, which was homogenized and incubated at 50 °C for 20 min. Afterward, 120  $\mu$ L of trichloroacetic acid at 10% was incorporated to the incubated mixture, followed by 410  $\mu$ L of distilled water and 100  $\mu$ L of ferric chloride. Finally, the absorbance was measured at 700 nm and results were expressed as gallic acid equivalents per milliliter (GAE/mL) according to the calibration curve prepared with the same standard.

### 2.7. Determination of mineral elements

The mineral content of the rambutan husk was determined. About 1 g of dried plant material was placed in a flask and a mixture of perchloric acid/nitric acid (1:3) was added (40 mL). This mixture was boiled to observe a color change, after that, the mixture was cooled with distilled water (20 mL) and filtered through Whatman 41 membranes. Distilled water was added to the mixture to reach 100 mL in a volumetric flask. Cu, Mn, Fe, Zn, Mg, K, Na and Ca elements were determined using a Varian AA-1275 atomic absorption equipment (Varian, Palo Alto, California, USA).

### 2.8. Identification of polyphenolic compounds by HPLC/ESI/MS analysis

The compounds obtained after Amberlite XAD-16 chromatography were analyzed by HPLC (Varian Prostar) with diode array detector (280 nm). About 1.8 mL of ethanolic fraction obtained after Amberlite XAD-16 chromatography were filtered (0.45  $\mu$ m membranes). The compounds separation was carried out in a Grace Denali c-18 column (5  $\mu$ m, 250 mm  $\times$  4.6 mm) at 30 °C. The mobile phase A was methanol (wash), B acetonitrile and C acetic acid 3% (initial 3% B and 97% C, 0–5 min 9% B and 91% C, 5–15 min 16% B and 84% C, 15–30 min 33% B and 67% C, 30–33 min 90% B and 10% C, 33–35 min 90% B and 10% C, 35–42 min 3% B and 97% C), flowed 1 mL/min, injection volume 10  $\mu$ L. Mass analysis was performed using a Varian 500-MS ion trap equipment, electrospray ionization (ESI), capillary voltage 90 V, negative mode ( $[M-H]^-$  *m/z*), mass acquisition range 100–2 000 *m/z*.

## 3. Results

### 3.1. Determination of total hydrolysable and condensed polyphenols

Polyphenolic content represented 58.2% of rambutan husks analyzed in this study. There was 457 mg/g dry matter in the content of total hydrolysable polyphenols. Moreover, the content of total condensed polyphenols determined after the extract purification was 125 mg/g dry matter.

### 3.2. ABTS antioxidant assay and ferric reducing antioxidant power

In this study, antioxidant activity of polyphenolic compounds of rambutan husks was determined by the half maximal

inhibitory concentration of ABTS-scavenging capacity (38.24 µg/mL). On the other hand, the value of ferric reducing antioxidant power recorded for polyphenolic compounds from rambutan husks was 0.203 GAE/mL.

### 3.3. Determination of mineral elements

The mineral content of rambutan husk not cultivated in Mexico was shown in Table 1. The presence of these minerals

**Table 1**

Mineral composition (mg/L dry matter) of rambutan peel.

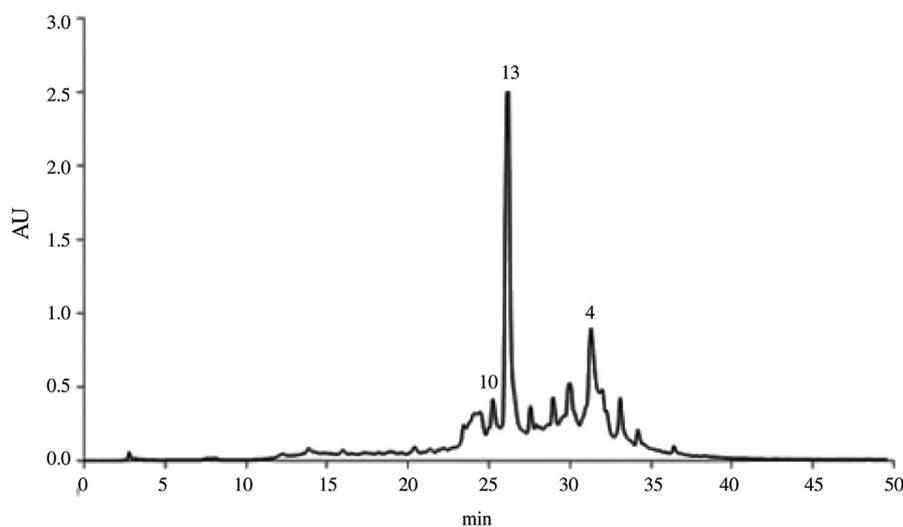
Mineral element	Rambutan husk
Cu	0.070 ± 0.001
Mn	0.14 ± 0.010
Fe	0.29 ± 0.020
Zn	0.080 ± 0.007
Mg	0.15 ± 0.020
K	0.57 ± 0.020
Na	0.04 ± 0.010
Ca	0.51 ± 0.010

provided added value to rambutan husk, as a source for the recovery of natural substances with polyphenolic content.

### 3.4. Identification of polyphenolic compounds by HPLC/ESI/MS analysis

The polyphenolic profile of rambutan husk cultivated in Mexico was determined by HPLC/ESI/MS. This approach consisted of the MS operating conditions: negative ionization mode, to achieve the identification of compounds present in the sample. The comprehensive evaluation of rambutan husk polyphenols allowed the identification of a total of 13 compounds. Figure 1 showed the separated compounds by HPLC, the main compounds reported for rambutan were identified, such as ellagic acid, corilagin and geraniin. For peaks assignment of these compounds, it could be seen in Table 2.

Among the classes of identified polyphenolic compounds in the sample, hydrolysable (poly)phenols were those more relevant. Anthocyanins, flavonoids and organic acids were also detected. The separated peaks by HPLC were identified by molecular weight using the VARIAN Work Station (version 2.0) database of the Autonomous University of Coahuila (FCQ-DIA-UAdeC). The identified compounds were shown in Table 2.



**Figure 1.** Chromatographic profile of main compounds in rambutan peel. (10) corilagin, (13) geraniin and (4) ellagic acid.

**Table 2**

Identification of phytochemical compounds in rambutan peel by HPLC/ESI/MS.

ID	Compounds	[M-H] <sup>-</sup> (m/z)	MS <sup>2</sup> (m/z)	Group
1	Apigenin	269	227, 197, 153, 151	Flavone
2	Pelargonidin	270	251, 228, 196, 116	Anthocyanin
3	Brevifolin carboxylic acid	291	247, 248, 203	Ellagitannin
4	Ellagic acid	301	257, 229, 185	Ellagitannin
5	p-Coumaroyl glucose	325	187, 163, 145, 119	Hydroxycinnamic acid
6	Vanillic acid hexoside	329	239, 168, 167	Hydroxybenzoic acid
7	Ellagic acid pentoside	433	301, 300	Ellagitannin
8	Vitisin A	560	508, 444, 442, 399	Anthocyanin
9	Apigenin arabinoside-glucoside	563	473, 443, 395, 353	Flavone
10	Corilagin	633	481, 301, 275	Ellagitannin
11	Castalagin/Vescalagin	933	915, 631, 451, 301	Ellagitannin
12	Galloyl-bis-HHDP-hexoside (Casuarinin)	935	657, 571, 463, 301	Ellagitannin
13	Geraniin	951	933, 301, 169	Ellagitannin
14	Unknown	979	935, 926, 862, 810	Unknown

#### 4. Discussion

For the total hydrolysable polyphenols, lower values were reported by Palaniasmy *et al.* [10] where the authors reported 393 mg/g of phenolic in rambutan husks aqueous extracts. This could be related to the total hydrolyzable polyphenolic content recorded for our samples after purification, which was 457 mg/g on dry weight. Similar results have been reported for rambutan husk not cultivated in Mexico; Maran *et al.* [20] reported a content of 402 mg/g of hydrolysable phenolic in rambutan husk extracts, Maisuthusakul *et al.* [21] also reported 203 mg/g of hydrolysable polyphenols in rambutan husk. The content of hydrolysable polyphenols detected in this study is due to the fact that rambutan husk is one of the sources with the highest polyphenolic content in nature [7,9]. Other authors [5] have reported higher contents of polyphenolic compounds in rambutan husk (702 mg/g) compared with the content reported in this study, the variation in polyphenolic content may be due to rambutan culture conditions in Mexico, which include climatic and soil conditions for rambutan growth (mainly in Chiapas, Mexico) [22], because these conditions have a direct influence on the synthesis of metabolites, such as polyphenols in plants. The polyphenol content reported in this study is relevant because there is no information about the quantification of rambutan husk cultivated in Mexico polyphenols. In case of condensed polyphenols content, some authors have reported the content of condensed phenols in rambutan husk extracts (not cultivated in Mexico). Dembitsky *et al.* [23] reported a condensed phenols content of 104 mg/g dry weight, while Oh *et al.* [24] reported a condensed phenols content of 13.3 mg/g dry weight in rambutan seeds. The color of rambutan peel cultivated in Mexico is red, because anthocyanins and procyanidins provide that characteristic red color [17,22,25]. This is the first study where the phenolic content of rambutan cultivated in Mexico including the condensed phenols content is reported. Therefore, the sum of the hydrolyzable phenols and condensed phenols, is considered the total phenol content present in rambutan husk cultivated in Mexico, the total value obtained was 582 mg/g dry matter.

For the ABTS-scavenging capacity was obtained a higher value than those previously reported by Ling *et al.* [5], when the antioxidant capacity of several Malaysian plants was evaluated using the same method. Differences could be attributed to chemical components in plants which depend on several environmental conditions as well as geographic origin of samples. Moreover, geraniin has been earlier reported as a phenolic compound present in rambutan husks, which has high free radical-scavenging, low pro-oxidant capability and the ability to inhibit carbohydrate hydrolyzing enzymes [10]. In this sense, for the presence of this ellagitannin in rambutan husks analyzed in the present study, we might consider this by-product as a good source of antioxidants and anti-hyperglycemic factors.

When minerals that are ingested in the diet can provide a chemo preventive effect against cancer [26]. It is worth mentioning that the potassium has been associated with beneficial effects [27], so its presence in the rambutan husk is relevant. In general, the identified minerals in this study have been reported in other sources that contain bioactive compounds such as grapes and grape peel [28]. The determined minerals in rambutan husk are of interest to the nutraceutical

and food industries, so the use of this plant material is a viable option for recovery of elements of interest and also for the recovery of added value bioactive compounds.

For the polyphenolic compounds identification, ellagitannins are polymeric structures including different numbers of galloyl and hexahydroxydiphenoyl units esterified with glucose. A total of 7 ellagitannins were tentatively identified for the first time in rambutan husk cultivated in Mexico. Three compounds of these ellagitannins have been already described in rambutan peel not cultivated in Mexico (compounds 4, 10, 13) [6,10], the compounds 3, 7, 11 and 12 were reported for the first time in rambutan husk (including varieties not cultivated in Mexico). Compounds 3 and 7 also have been identified in other plant sources and their biological potential is important [29,30], so the presence of compounds 3 and 7 in rambutan husk is relevant. In general, the presence of ellagitannins is relevant since they have an important biological potential, such as the compounds 11 and 12 [31].

Compound 6 was identified as hydroxybenzoic acid. The presence of these compounds has been described in plant sources like pomegranate peel, and its antioxidant potential has been reported [29,30,32], so its presence in rambutan peel cultivated in Mexico provides added value to this material as a source for the recovery of antioxidant compounds. Compound 5 was identified as a hydroxycinnamic acid which has been associated with the pigmentation of rambutan husk [33].

Compounds 1, 2, 8 and 9 were identified as flavonoids. Flavonoids are associated with the natural pigmentation of rambutan husk, including red, brown, violet colors [24,34,35], which justifies the identification of these compounds. These compounds have biological potential, such as antioxidant capacity and antimicrobial activity [36,37].

In conclusion, rambutan not cultivated in Mexico (husk) represents an important source for the recovery of added value compounds with antioxidant activity, which have potential applications as bioactive antioxidant agents for the treatment of diseases. The polyphenolic analysis of this plant material allowed the identification of 13 compounds, which may have application in the medicine filed.

#### Conflict of interest statement

The authors declare there is no conflict of interest.

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