Antioxidant and antibacterial extracts from rambutan (*Nephelium lappaceum*) skins: Exploring the Potential of Transforming Agricultural Byproducts into Functional Supplements

Extractos antioxidantes y antibacteriales de las cáscaras del rambután (*Nephelium lappaceum*): explorando el potencial de transformación de subproductos agroindustriales en suplementos funcionales

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Keywords

Rambutan; antioxidants; antibacterial; *Nephelium lappaceum*; functional supplements.

Abstract

Rambutan (*Nephelium lappaceum* L.) is a tropical fruit characterized by its oval shape and hairy skin, primarily valued for its juicy pulp. The peels, constituting 45% of the fruit's weight, are a source of valuable compounds like geraniin, ellagic acid, and quercetin. These peels possess antimicrobial properties effective against various bacteria, making them suitable for food preservation and packaging. Additionally, rambutan extracts hold promise as supplements in animal feed, enhancing growth and reducing methane production. This research delves into the antioxidant and antimicrobial attributes of diverse rambutan varieties. The skin (exocarp) of rambutan fruits from three Costa Rican cultivars -Creole, Rongrein, and Yellow- were collected and processed. Total polyphenolic content (TPC), proanthocyanidins, antioxidant activity, geraniin content, and antimicrobial activity were determined for the three varieties. Also, proanthocyanidin-enriched fractions from rambutan extracts were generated and analyzed. The results revealed TPC and antioxidant activity variations among different rambutan varieties and harvest years. All rambutan extracts displayed antimicrobial activity. In conclusion, the research underscores the rich antioxidant content in rambutan peels, irrespective of the variety, and underscores their potential for use in both human and animal nutrition due to their chemical composition.

Palabras clave

Rambután; antioxidantes; antibacterial; *Nephelium lappaceum*; suplementos funcionales.

Resumen

El rambután (*Nephelium lappaceum L.*) es una fruta tropical caracterizada por su forma ovalada y su cáscara peluda, de la cual principalmente se aprovecha su pulpa jugosa. Las cáscaras, que constituyen el 45% del peso de la fruta, y son una fuente de compuestos valiosos como la geranina, el ácido elágico y la quercetina. Estas cáscaras poseen propiedades antimicrobianas efectivas contra varias bacterias, lo que las hace adecuadas para la conservación y el envasado de alimentos. Además, los extractos de rambután prometen ser suplementos en la alimentación animal, mejorando el crecimiento y reduciendo la producción de metano. Esta investigación se adentra en los atributos antioxidantes y antimicrobianos de diversas variedades de rambután. Para este efecto, se recolectaron y procesaron las cáscaras (exocarpo) de frutas de rambután de tres variedades costarricenses: Criolla, Rongrein y Amarillo. Se determinó el contenido total de polifenoles (TPC), proantocianidinas, actividad antioxidante, contenido de geranina y actividad antimicrobiana de las tres variedades. Además, se generaron y analizaron fracciones enriquecidas de proantocianidinas a partir de extractos de rambután. Los resultados revelaron variaciones en el TPC y la actividad antioxidante entre las diferentes variedades de rambután y los años de cosecha. Todos los extractos de rambután mostraron actividad antimicrobiana. En conclusión, la investigación resalta el rico contenido antioxidante en las cáscaras de rambután, independientemente de la variedad, y subraya su potencial uso tanto en la nutrición humana como animal debido a su composición química.

Introduction

The rambutan, scientifically known as *Nephelium lappaceum* L., is a tropical fruit classified within the Sapindaceae family [1]. Rambutan is a hairy oval-shaped fruit. It is composed of peel, pulp, seed, and embryo. The peels of this fruit are green, yellow, or red. The pulp is used for human consumption, and other parts are generally considered waste. Rambutan peels account for 45% of the fruit weight [2], and the peels are rich in geraniin, corilagin, rutin, ellagic acid, quercetin, and total phenolic compounds (TPC) [3]. Geraniin is an ellagitannin used as a food additive. Several health benefits have been associated with geraniin consumption [4]. It is a strong antioxidant compound, and it is reported to have antihypertensive, antiviral, antidiabetic, antihyperglycemic, and hepatoprotective activities [5].

Chemical composition varies depending on the variety utilized [6], plant maturity [7], and climate. Rambutan is a fruit from Asia, and it is produced in several tropical countries all over the world. The production of this fruit in Costa Rica is concentrated in the southern region. The commercialized rambutan fruit is a mixture of the available varieties.

Rambutan peel extracts are antimicrobials. Phuong *et al* [3] found antimicrobial activity against *Salmonella enteritidis, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Lactobacillus plantarum, Vibrio campbellii,* and *Listeria monocytogenes.* Interestingly, the same authors found no difference between the antimicrobial activity against antibiotic-resistant and non-resistant strains of *S. enteritidis* when rambutan peel extract of 100 µg GAE/mL concentration or higher is evaluated. Polyphenolic compounds are reported to be active against antibiotic-resistant bacteria [8] because their mechanism of action is different. The antimicrobial properties of rambutan extracts have been utilized for food preservation and food packing [9, 10]. *N. lappaceum* extracts have also been studied as an animal feed supplement, with no negative impact on animal microbiota composition [11]. Nonetheless, it reduces the methane production in beef and promotes better growth performance and immune response in Nile Tilapia [12], and catfish [13]. In this study, a preliminary assessment of the main antioxidant compounds and their antioxidant and antimicrobial activity was done. Our aim is to study the properties of different varieties from two different harvest times, as potential supplements for human or animal feeding applications.

Materials and methods

Biological materials

We collected the exocarp of the fruit from three rambutan cultivars (*Nephelium lappaceum* L) produced in Costa Rica. Creole, Rongrein, and Yellow varieties were sampled. Fruit was collected from the Pérez Zeledón canton in the province of San Jose, located at latitude 9°18'31.84" N, longitude 83°40'14.54" W. Fruits were collected during 2013 (samples R1), 2014 (R2), and 2015 (R3). The sampling region was selected because most rambutan producers are located there. The exocarp (skin) was separated from the pulp and the seed. Samples were stored in coolers after collection, immediately transported to the lab to be frozen, and then dehydrated in a Freeze-dryer 2.5 L plus (from Labconco Corp, MO, USA). The dried samples were ground in a medium-sized Wiley blade mill to a 1 mm (from Thomas Scientific, NJ, USA).

Optimization of the extraction procedure

The solvent type and number of extraction cycles were optimized. The solvents to be tested include acetone:methanol:5% HCl (4:4:2), acetone:5% HCl (7:3), acetone:ethanol:5% HCl (4:5:1), and 5% HCl in 95% ethanol. A composite sample comprising all cultivars in the same proportion was used. 75 mg of dry and ground sample was extracted with 3x3mL of the solvent.

In each extraction, the sample was sonicated with the solvent for 5 minutes. Then. the tubes were centrifuged at 400 g for 5 minutes, and the supernatant liquid was decanted. The three extractions were combined and adjusted to 10 mL final volume and analyzed using Folin-Ciocalteau´s protocol, as described below.

Then, optimal extraction cycles were optimized with the most efficient solvent. 5 test tubes, containing 75 mg of the sample were extracted using 1x2mL, 2x2mL, 3x2mL, 4x2mL, and 5x2mL, respectively. All tubes were adjusted to 10 mL and analyzed using Folin-Ciocalteau´s protocol.

Determination of Total Polyphenolic Compounds (TPC)

Peel material was directly extracted with the optimized extraction procedure. 75 mg of previously lyophilized and ground samples were extracted using three 3 mL aliquots of 95% ethanol acidified to 5%. The procedure was repeated 3 times. Subsequently, the combined 9 mL obtained was mixed to a final volume of 10 mL in an appropriate flask using acidified ethanol.

Three replicates of this procedure were prepared. Finally, the assessment of the TPC was conducted using the Folin-Ciocalteu colorimetric method, as previously described in our report [14]. Briefly, 30 µL of each of the previously prepared samples was mixed with 200 µL of water in a 96-well microplate for analysis. Subsequently, 15 µL of Folin-Ciocalteu reagent and 50 µL of a 20% sodium carbonate solution were added to the microplate. The plate was then incubated for 20 minutes with agitation at 40 °C in a Synergy HT Multi-Detection Microplate Reader (BioTek Instruments). After the incubation, the absorbance was measured at 755 nm, against standard solutions of 0.000, 0.020, 0.040, 0.060, 0.080, and 0.120 mg/mL gallic acid.

Rambutan proanthocyanin standards (rPAC) were also analyzed using Folin-Ciocalteau´s method. 1 mg of rPAC powdered was dissolved into 1 mL of ethanol and analyzed as described before.

Determination of antioxidant activity by the DPPH method

The antioxidant activity of DPPH (2,2 Diphenyl-1-picrylhydrazyl) was determined following the method described by Bondet [15], in a 96-well microplate. 30 µL of methanol:water (80:20) was used as blank. Wells designated as standards (STD) utilize 30 µL of a 0.0215 mg/mL gallic acid standard, and the wells designated for the samples (SPL) were filled with 30 µL of the sample, instead. Subsequently, 270 µL of the 0.042 mg/mL DPPH solution was added to each well, and the plate was placed in the microplate reader. After 30 minutes, the absorbance was measured at a wavelength of 515 nm.

Determination of antioxidant activity by ORAC method

Oxygen radical absorbance capacity was determined using the method described by Brescia [16]. 150 µL of $4x10^{-6}$ mM sodium fluorescein solution was added to all the wells. 25 µL of the samples were added to the wells. Then, the plate was incubated for 20 minutes at 37 °C in the microplate reader. After this incubation period, the plate was removed from the reader, and 25 µL of 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) solution 153 mM was immediately added to all the experimental wells. The microplate was placed back into the reader, and readings were taken every minute for a total of 60 readings per well. Fluorescence was measured at 485 nm (excitation) and 528 nm (emission), against 0, 25, 50, 75, and 100 µM Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard curve. 75 mM phosphate buffer (pH 7.4) was used as a solvent for all solutions and blank.

Preparation of proanthocyanidin-enriched fractions of rambutan (rPAC)

Seven grams of dried rambutan sample were weighed, and five extractions of 50 mL each were performed using 95% ethanol. The obtained extracts were filtered and concentrated using a rotary evaporator under reduced pressure and 40°C. 5 mL ethanol 95% was added and centrifuged at 0°C and 11,000 rpm for 10 minutes. After centrifugation, the supernatant fluid was added to a column containing Sephadex LH-20, and eluted by adding ethanol: methanol in a 1:1 (v/v), and then acetone:water in a 7:3. Three fractions were collected, concentrated, freeze-dried, and analyzed for proanthocyanidins (PAC) (method described below). The fraction with the highest PAC concentration is considered the rPAC. They are stored at -18°C until use.

Determination of Proanthocyanidins

Three replicates and three repetitions each were done. 70 µL of sample and 210 µL of 0.1% (w/v) DMAC solution were mixed into each well. Immediately, the plate was placed into the reader and stirred for 10 seconds at 600 revolutions per minute (rpm). Then, the absorbance was measured at 640 nm every 30 seconds for one hour. Blank solution (80% ethanol), and standard curves of 0–0.03 mg/L 4'-O-methyl-gallocatechin, and 0–0.16 g/L rPAC were utilized as references.

Geraniin quantification

Geraniin was quantified using a modification of a previously reported method [17]. An LC20 HPLC-DAD chromatographer (from Shimadzu Corp., Kyoto, Japan) equipped with a Dionex Acclaim 120, C18-column (150 mm 4,6 mm i.d., 5 µm) was used to quantify the amount of geraniin. 20µL of the sample was utilized. The mobile phase consisted of (A) 0,1% formic acid and (B) acetonitrile with a gradient from 0-28 min, 0-20% B in A, 28-34 min, and 20-70% B in A. The flow rate was 0,8 mL/min. The detection was performed using 280 nm.

Determination of Antimicrobial Activity

Antibacterial activity was determined using the Kirby-Bauer disc diffusion method as previously reported [18]. Each disc was impregnated with 50 µL of each of the extract. 30 µg/mL chloramphenicol has been used as the positive control, and water:acetone (7:3) as the negative control. *Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 25923*, and *Bacillus subtilis ATCC 6633* were utilized to test susceptibility.

Results and discussion

Optimization of the extraction procedure

Figure 1(A) shows the results of the solvent choice to extract rambutan shells. All the solvents tested were enriched with HCl 5% to increase their stability. Rambutan extracts are stable at a pH lower than 3 [19]. Acetone, short alcohols, and water were components included in the potential extraction solvents tested. A significant difference is observed in ethanol 95% over the other solvent mixtures.

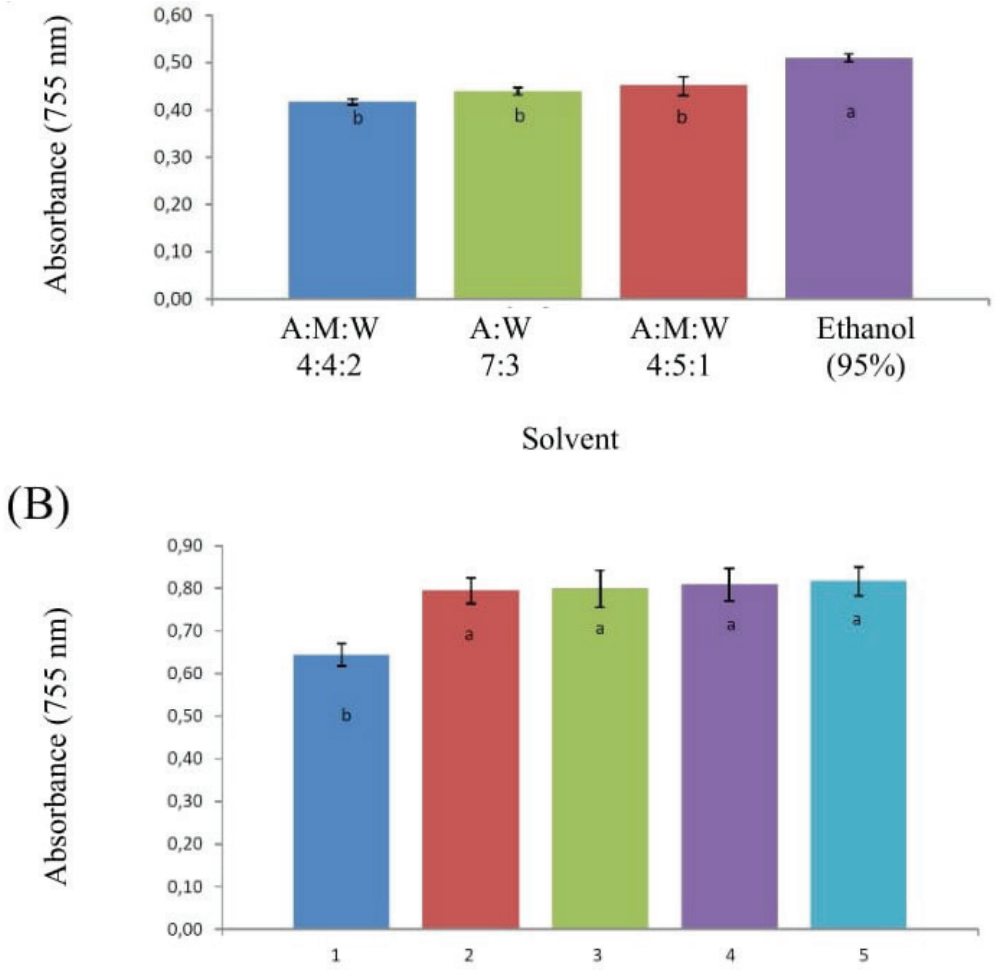

Figure 1. Optimal extraction conditions. (A) Solvent selection. A: Acetone, M: methanol, and W: water. All solvents contain 5% HCl (B) Number of extraction cycles. Compact display letters represent Tukey´s test. α=0.05, n=3.

Figure 1(B) also represents the number of extraction cycles. Every cycle represents 2 mL of solvent, sonication, and centrifugation. The sonication step helps to destroy cell structures and accelerates the extraction process. This method is fast and utilizes small solvent volumes. According to Das [20], ultrasound-assisted extraction efficiency is slightly lower than maceration or Soxhlet extraction. However, several cycles usually help to increase efficiency to a similar level. Also, thermal treatment, such as a Soxhlet can induce the degradation

of thermolabile metabolites, The Rambutan extraction process shows no significant differences after the second cycle. The metabolites extraction process is very fast and efficient for rambutan, respecting other matrices. E.g., 3 extraction cycles are required for both corn (*Zea mays*) [14] and ber (*Ziziphus mauritiana*) [21].

TPC and antioxidant activity of crude extracts

Previously, some compounds have been identified in the literature as main components for rambutan extracts [22, 23] geraniin, corilagin, rutin, ellagic acid, gallic acid, and some derivatives. Folin-Ciocalteu´s test for TPC reacts with phenolic substances (and some other reducing compounds), while the antioxidant tests are based on two different mechanisms: hydrogen atom transfer (such as in the ORAC method), and electron transfer (such as in the DPPH method) [24]. Phenolic compounds are considered the main contributors to the antioxidant capacity of

rambutan extracts [22]. Figures 2(C) and 2(F) show the relationship between ORAC vs TPC and DPPH vs TPC. Pearson coefficients are 0.6981, and 0.5240, respectively. The correlations are low compared to other types of samples such as some teas, where the Pearson coefficient takes values ranging from 0.99 to 1.00 [24]. Individual contributions of rambutan components have been assessed in the past and found significantly different. Geraniin, corilargin, and ellagic acid have 32-65% antioxidant capacity of gallic acid [23]. Then, low correlations can be explained by different distributions of phenolic compounds.

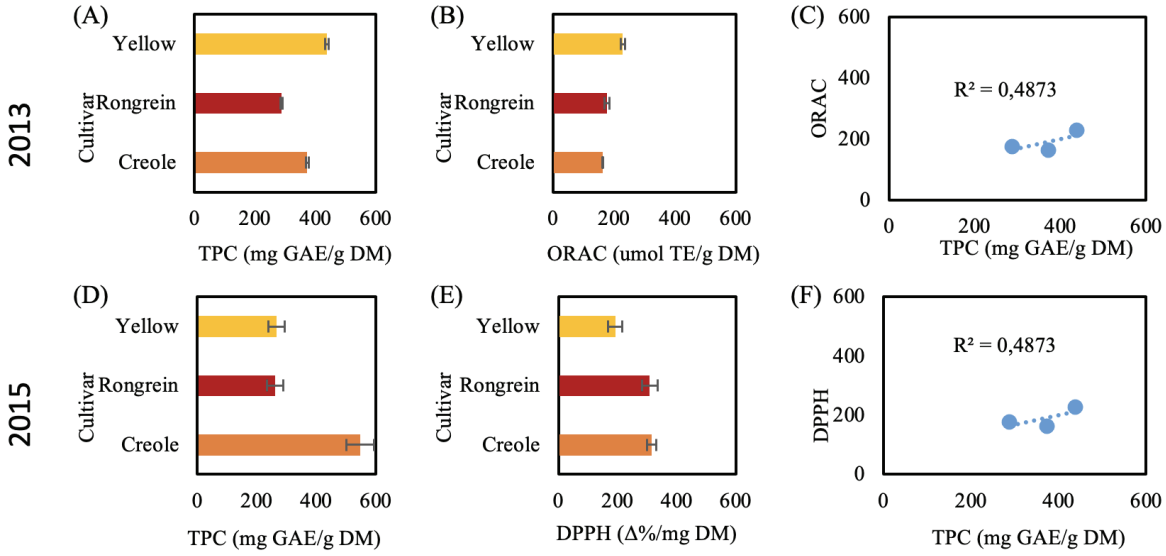

Figure 2. Total Phenolic Compounds (TPC), antioxidants (DPPH and ORAC), and Proanthocyanidins (PAC) from selected rambutan samples.

Samples harvested during 2013 and 2015 (Figures 2(A) and 2(D)) have different TPC content. Yellow variety showed the highest from the first group (438 mg GAE/g) but decreased to 266 mg GAE/g in 2015. The Creole variety was the second highest during the 2013 harvest (373 mgGAE/g) and then increased to 547 mgGAE/g, which is the highest value. Rongrein variety just suffers small variations in TPC concentration, being the middle value for both sets of samples (288 and 261 mgGAE/g).

Proanthocyanidins-enriched extracts

The rPAC fraction is prepared by purifying PACs from the crude extract. This procedure leads to a "self-fruit" standard, in a similar way to previous reports [25]. rPAC standard is more representative of the heterogenicity of PACs present in rambutan than a commercial reference such as 4-MGC. In this work, we quantified TPC content, DPPH antioxidant activity, and PACs content from the rPAC fraction extracted from the three varieties included in this study. Results are shown in Figure 3.

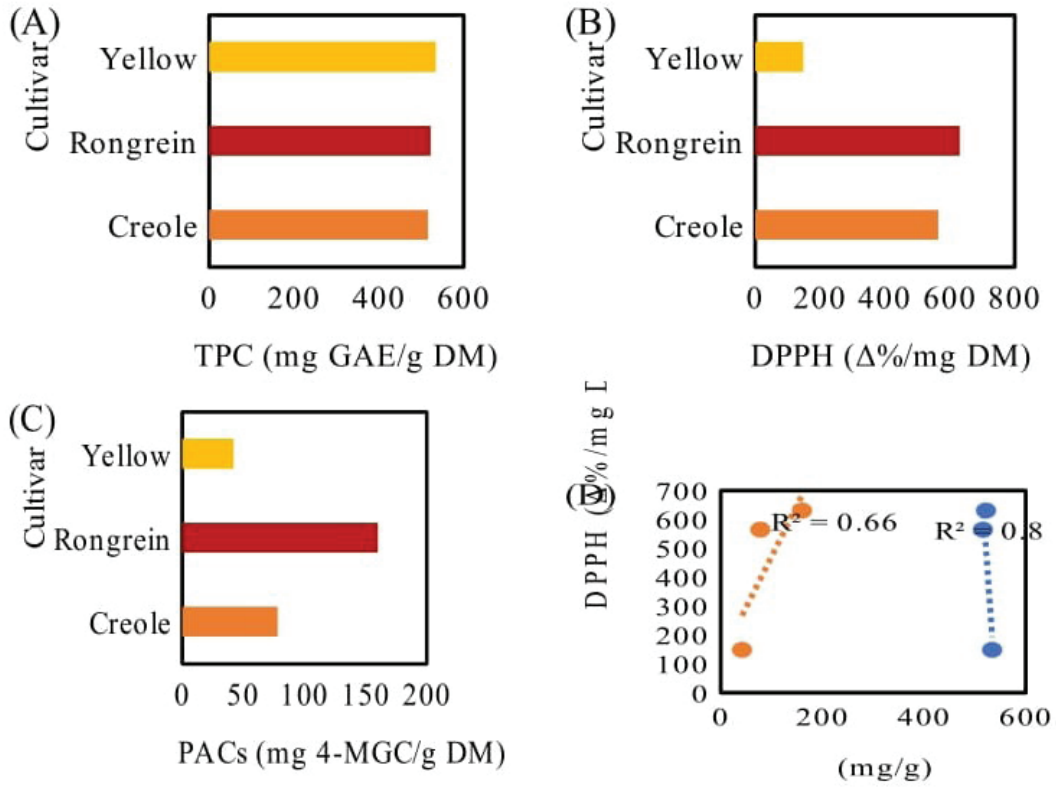

Figure 3. Total phenolic compounds (TPC), antioxidants (DPPH), and proanthocyanidins (PAC) from rPACs.

TPC of the rPACs of the three varieties ranges from 516-534 mg GAE/g. Values are very consistent probably because rPACs are supposed to be near 100% proanthocyanidins. Flavonoids such as PACs and other phenolic compounds are the most abundant pigments in fruits, including rambutan peels [26]. Then, more abundant conjugated, or delocalized aromatic systems can be found in red varieties (such as rongrein and creole) than in the yellow varieties.

Figure 3(D) shows the Pearson coefficient for DPPH vs TPC or PACs. The correlation between PACs and antioxidant activity is clearer than in the crude extracts. The lack of other contaminants (because of the purification of rPACs) seems to help to establish a better relationship. However, PACs from the three varieties are different in chemical nature.

Geraniin analysis

Some ellagitannins have particular attention because they are reported to have several bioactivities. Geraniin, an ellagitannin previously reported in *N. lappaceum* skins has been selected to be quantified. Figure 4 shows the composition of the three varieties included in this study.

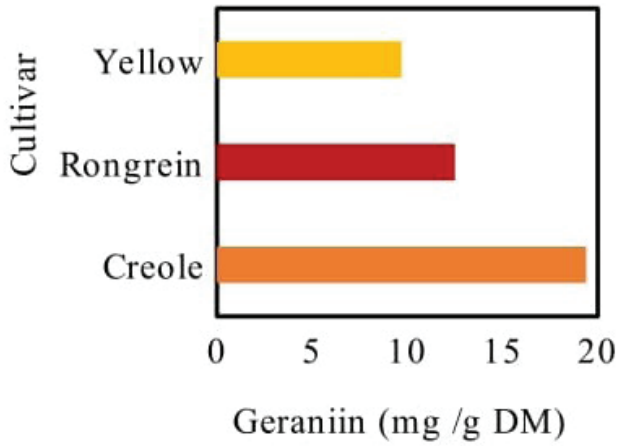

Figure 4. Geraniin composition of a sample of three rambutan varieties.

Concentrations of geraniin range from 9.7 to 19.4 mg/g. Creole variety reached the highest concentration.

Antimicrobial activity

Figure 5 shows the results for the antimicrobial activities of rambutan extracts against four bacterial strains.

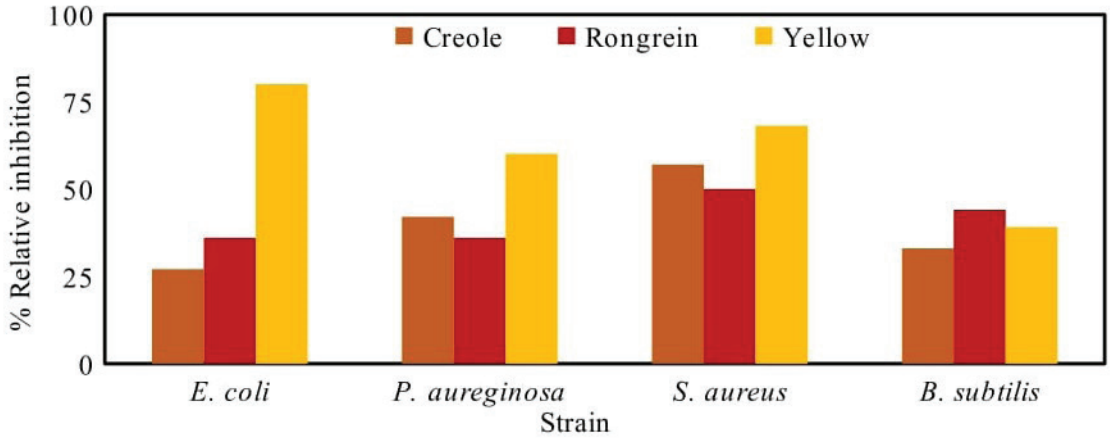

Figure 5. Antimicrobial activity of 50 µL of rambutan extracts. 30 µg chloramphenicol has been used as positive control, and water:acetone (7:3) as negative control. n=3. Error bars represent standard deviations. Letters on top of columns represent Tukey test grouping (samples containing the same letter belong to the same group), per bacteria tested.

The three varieties have antibacterial activity against the four microorganisms tested. Bacteria included two gram-positive and two gram-negative microorganisms. Also, the yellow variety of rambutan showed the highest antimicrobial activity, reaching 80% relative inhibition for *E. coli* and 60 and 68% for *P. aeruginosa* and *S. aureus,* respectively. These results are similar to those found in other publications [3, 23], in which the antibiotic activity is related to the phenolic content of the extracts.

Conclusions

The three varieties of rambutan included in this study (Creole, Yellow, and Rongrein) are antioxidant-rich in their peels. The concentration between varieties does not keep a pattern between the samples of two different years included in this study. TPC composition is 250-550 mg GAE/g in all the samples, and 10-20 mg/g geraniin. All the extracts have shown antimicrobial properties and antioxidant activity.

Peels from Yellow, Rongrain and Creole rambutan are suitable for both human and animal supplementation according to the chemical composition.

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