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The Anti-Hypertensive and Hypoglycemic Potential of Bioactive Compounds Derived from *Pulasan* Rind

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Abstract: *Pulasan* (*Nephelium mutabile Blume*) is an underutilized fruit native to tropical countries, including Malaysia, Thailand, and Indonesia. To date, the medicinal potential of *pulasan* remains unexplored, although this fruit shares the same genus with the well-known *rambutan* (*Nephelium lappaceum*). Therefore, the current study aims to examine the antioxidant properties of different parts of *pulasan* (flesh, rind, and kernel) and investigate the bioactive profile, anti-hypertensive and hypoglycemic properties of *pulasan* rind. *Pulasan* were extracted using different solvents, including distilled water, methanol, and ethanol. The antioxidant capacity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox equivalent antioxidant capacity (TEAC), and ferric reducing antioxidant power (FRAP), and the antioxidant component was identified by total flavonoid content (TFC) and total phenolic content (TPC). The bioactive profile of *pulasan* rind was characterized by high-performance liquid chromatography (HPLC). The anti-hypertensive and hypoglycemic properties of *pulasan* rind were determined with angiotensin-converting enzyme (ACE) assay and alpha-amylase inhibition assay, respectively. Emerging findings revealed that *pulasan* rind exhibited the highest antioxidant capacity (DPPH, TEAC, and FRAP) in all extraction solvents and antioxidant components (TPC and TFC) in ethanolic extract. The ethanolic extracts of *pulasan* rind also had higher ACE and alpha-amylase inhibition activities than the distilled water extracts. Geraniin, chlorogenic acid, catechin, corilagin, syringic acid, and naringenin of *pulasan* rind may function as anti-hypertensive agents.

Keywords: antioxidant capacity; *Nephelium mutabile Blume*; ACE inhibitory; alpha-amylase inhibitory; bioactive compounds

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1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are harmful free radicals that attack macromolecules and cause a cellular injury that disrupts normal cellular activities. Numerous studies suggest that chronic cellular injury is highly linked to the development of non-communicable diseases (NCDs), including various types of cancers, hypertension, and diabetes mellitus [1]. Having a sufficient amount of bioactive compounds from fruits and vegetables could delay the progress of cellular injury, which may be beneficial in NCDs prevention [2].

Pulasan, *rambutan*, *salak*, *durian*, and *bacang* are among the underutilized fruits native to tropical countries, including Malaysia, Indonesia, and Thailand [1,3]. A large body of literature claims that these fruits contain appreciable bioactive compounds [1,3]. *Rambutan*, for instance, was previously reported for its significant amount of ellagic acid, corilagin, and geraniin. These bioactive compounds are claimed to be accountable for the reported anti-inflammation, anti-diabetic, and anti-cancer properties of *rambutan* [4].

Pulasan (*Nephelium mutabile Blume*) is an underutilized fruit that highly resembles *rambutan* (*Nephelium lappaceum*). It has a dark reddish or yellowish rind, a white to yellowish flesh, and an oblong-shaped greyish-brown kernel [5]. The flesh is the only edible portion of this fruit, while the rind and kernel are often discarded as solid wastes [6]. Although the rind is one of the by-products of *pulasan*, a previous study by Chan et al. [7] revealed that it could induce apoptosis in HT29 human colon cancer cells. To date, only a few studies have reported the antioxidant properties of *pulasan* (antioxidant capacity and antioxidant components) and its by-products. Moreover, little is known about the bioactive profile, or the anti-hypertensive and hypoglycemic potential of this fruit and its by-products. Therefore, this research aims to examine the antioxidant properties of *pulasan* parts (flesh, rind, and kernel) with three types of extraction solvents (distilled water, methanol, and ethanol) and investigate the bioactive profile and anti-hypertensive and hypoglycemic properties of *pulasan* rind.

2. Methodology

2.1. Preparation and Extraction of Samples

Pulasan fruits of uniform maturity and similar size were purchased from Selangor Fruits Valley, Malaysia. Upon arriving at the laboratory, the *pulasan* was cleaned under running tap water to remove physical dirt. All cleaned fruits were then manually sorted into three different parts (flesh, rind, and kernel). All parts were subject to the freeze-drying process for preserving the potential bioactive compounds. The freeze-dried samples were ground into powder and kept in three separate opaque containers at −80 °C prior to analysis.

Extraction with distilled water, pure ethanol, and pure methanol was carried out in the ratio of 1:25 by incubating in an orbital shaker that was preset at 200 rpm (revolutions per minute) at 50 °C for 120 min (min). Whatman paper No. 542 was used to filter all the extracts, and the filtrates were kept at −20 °C until further analysis [8].

2.2. Determination of Antioxidant Properties of Sample Extracts

2.2.1. Total Flavonoid Content (TFC)

The TFC was investigated with the method of Shin et al. [9]. A 1.0 mL sample extract was diluted with 4.0 mL distilled water. Reagents were added in the following order: 0.3 mL of 5% sodium nitrite (5 min incubation at room temperature (R.T.)); 0.3 mL of 10% aluminum chloride hexahydrate (6 min incubation at R.T.); and 2.0 mL of 1 mol/L sodium hydroxide. Subsequently, distilled water was added to 10.0 mL. The absorbance was read at 510 nm. Results were presented as catechin equivalents per gram (mg CE/g) of dry sample.

2.2.2. Total Phenolic Content (TPC)

The TPC was quantified based on Ismail, Marjan, and Foong [10]. In brief, 0.1 mL of the sample extract was added to 0.75 mL of the Folin–Ciocalteu reagent, which had undergone 10-fold dilution for 5 min. Subsequently, 0.75 mL of 60 g/L sodium bicarbonate solution was added, followed by incubation for 90 min in the dark at R.T. The absorbance was measured at 725 nm. Results were presented as gallic acid equivalents per gram (mg GAE/g) of dry sample.

2.2.3. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was evaluated according to Enujiugha et al. [11]. In brief, 3.8 mL of 0.1 mM DPPH solution was added into 0.2 mL of the sample extract and placed in the dark at R.T. for 30 min. The absorbance was measured at 517 nm. The percentage of radical scavenging activity (RSA) was calculated as follows:

$$\% \text{ RSA} = \left(1 - \frac{\text{Ab sample}}{\text{Ab control}}\right) \times 100\%$$

where

Ab_{sample} = the absorbance of sample extract;
$Ab_{control}$ = the absorbance of extraction solvent.

2.2.4. Ferric Reducing Antioxidant Power (FRAP)

The FRAP reagent was prepared freshly by adding 300 mmol/L acetate buffer with pH 3.6, 20 mmol/L iron (III) chloride hexahydrate and 10 mmol/L TPTZ in 40 mmol/L HCl in a ratio of 10:1:1 (*v*/*v*) [8]. A total of 1.5 mL of FRAP reagent was added into the 0.05 mL sample and 0.15 mL distilled water. After incubating for 4 min at R.T., the absorbance was read at 593 nm. The results were presented as micromoles of iron (II) equivalent per gram (μmol Fe (II)/g) of dry sample.

2.2.5. Trolox Equivalent Antioxidant Capacity (TEAC)

The TEAC assay was quantified as described by Tan et al. [8]. In short, 7 mmol/L ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and 2.45 mmol/L potassium persulfate were mixed, followed by standing in the dark at R.T. for 16 h in order to obtain the ABTS solution. The ABTS solution was pre-diluted with the respective extraction solvent to reach an absorbance of 0.700 ± 0.050 before use. The sample was also diluted to provide the range of 20–80% inhibition of blank absorbance. A 0.05 mL diluted sample was added into 1.9 mL of diluted ABTS solution, followed by 6 min incubation. After that, the absorbance was measured at 734 nm. The results were presented as micromoles of Trolox equivalents per gram (μmol TE/g) of dry sample.

2.3. Angiotensin-Converting Enzyme (ACE) Inhibition Assay

The ACE inhibitory activity was determined as described by Abdullah et al. [12]. A 0.1 M sodium borate buffer (pH 8.3) containing 0.3 M sodium chloride (NaCl) was made to prepare 0.1 U/mL ACE and 5 mM substrate hippuryl-L-histidyl-L-leucine (HHL) solution. In brief, 0.2 mL of 5 mM HHL solution was mixed with 0.08 mL of sample and incubated for 3 min at 37 °C. Thereafter, 0.02 mL of 0.1 U/mL ACE was added to initiate the reaction for 30 min at 37 °C. Subsequently, 0.25 mL of 1N hydrochloric acid (HCl) was added to terminate the reaction. Next, 1.7 mL ethyl acetate was added, and the mixture was centrifuged for 15 min at 800× *g*. A total of 1.0 mL of distilled water was added after removing ethyl acetate by evaporation. Absorbance was measured at 228 nm using a spectrophotometer. The percentage of inhibition was calculated as follows:

$$\% \, I = 1 - \left(\frac{Ab_{\,ACE \,\&\, HHL} - Ab_{\,ACE \,\&\, sample}}{Ab_{\,ACE \,\&\, HHL} - Ab_{\,HHL}} \right) \times 100\%$$

where

$Ab_{ACE \,\&\, HHL}$ = the absorbance of angiotensin-converting enzyme and substrate;
$Ab_{ACE \,\&\, sample}$ = the absorbance of angiotensin-converting enzyme and sample extract;
$Ab_{\,HHL}$ = the absorbance of substrate.

2.4. Alpha-Amylase Inhibition Assay

The alpha-amylase inhibitory activity was determined based on Gonçalves, Lajolo and Genovese [13]. Ice-cold distilled water was used to produce 4 U/mL porcine pancreatic alpha-amylase enzyme solution, while the substrate was 0.5% *w*/*v* potato starch in 20 mM phosphate buffer at pH 6.9 containing 6.7 mM sodium chloride. Sample extract of 0.04 mL, 0.16 mL of distilled water, and 0.40 mL of substrate solution was mixed. Subsequently, 0.20 mL of enzyme solution was added into the mixture to initiate the reaction for 3 min at 25 °C. After that, 0.1 mL of dinitrosalicylic acid (DNS) color reagent solution (96 mM 3,5-dinitrosalicylic acid, 5.31 M sodium potassium tartrate in 2 M sodium hydroxide) was added to the mixture and placed in a water bath at 85 °C for 15 min. Thereafter, the mixture

was diluted with 0.9 mL of distilled water. Absorbance was measured at 540 nm. The percent inhibition was calculated as follows:

$$\%\,I = 1 - \left[\frac{(Ab_{\text{enzyme + solvent}} - Ab_{\text{solvent}}) - (Ab_{\text{enzyme + sample}} - Ab_{\text{sample}})}{\left[(Ab_{\text{enzyme+solvent}} - Ab_{\text{solvent}}) \right]} \right] \times 100\%$$

where

$Ab_{enzyme + solvent}$ = the absorbance of porcine pancreatic alpha-amylase solution and extraction solvent;

$Ab_{solvent}$ = the absorbance of extraction solvent;

$Ab_{enzyme + sample}$ = the absorbance of porcine pancreatic alpha-amylase solution and sample extract;

Ab_{sample} = the absorbance of sample extract.

2.5. Identification of Bioactive Compounds

Bioactive compounds in the *pulasan* rind extract were quantified using HPLC [14]. In brief, the ethanol extract was separated by using a reversed-phase Zorbax Eclipse Plus C18 column (250 × 4.6 mm, I.D. 5 µm, Agilent). A gradient elution was generated with 0.5% (*v*/*v*) acetic acid (solvent A) and 100% methanol (solvent B). A constant flow rate of 1.0 mL/min was used. The linear gradient profile was set as: 100% A and 0% B at the start, 10% A and 90% B at 20–25 min, and finally 100% A and 0% B at 30 min. A 20 µL sample extract was injected into the C18 column at R.T. The bioactive compounds were detected at 280 nm. Identification was performed by comparing the retention time generated by standards and the rind extracts. The standard of each identified phenolic compound was dissolved in the extraction solvent to obtain five concentrations (20, 40, 60, 80, and 100 µg/mL) for standard calibration curves. The bioactive compounds in the rind extracts were calculated by referring to the standard calibration curve.

2.6. Statistical Analysis

Data were analyzed using the IBM Statistical Package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Results were presented as mean ± standard deviation (S.D.) of triplicates. In addition, one-way analysis of variance (ANOVA) with Tukey's post-hoc test was used to determine mean differences between flesh, rind, and kernel of *pulasan*. The correlations of all studied variables were determined using Pearson's correlation test. Statistical significance was set at the *p*-value of less than 0.05 ($p < 0.05$).

3. Results and Discussion

3.1. Quantification of Antioxidant Components in Different Parts of Pulasan

Table 1 summarizes the antioxidant properties of different parts of *pulasan*. All samples were extracted with distilled water, pure methanol, and pure ethanol. TFC was in the range of 0.35 ± 0.03 mg CE/g (water extract of flesh) to 68.98 ± 6.71 mg CE/g (ethanolic extract of rind). The rind of *pulasan* exhibited the highest TFC regardless of the extraction solvents. Emerging findings revealed that the alcoholic extracts of *pulasan* rind had significantly higher ($p < 0.05$) TFC than its flesh and kernel, signaling that pure alcohol was a better extraction solvent compared to distilled water. Although the solvent polarity of methanol and ethanol are comparable [15], findings in the current study revealed that the ethanolic rind extract (68.98 ± 6.71 mg CE/g) recorded a higher TFC than the methanolic rind extract (40.75 ± 1.45 mg CE/g). Coincidentally, a recent review of Chaves et al. [16] also supports the notion that ethanol is more efficient in extracting flavonoids compared to pure water and methanol.

Table 1. The antioxidant properties of rind, fruit, and kernel of *pulasan* in different extraction solvents.

Extraction Solvent	Part of *Pulasan*	TFC (mg CE/g)	TPC (mg GAE/g)	DPPH (% RSA)	TEAC (μmol/g T.E.)	FRAP (μmol/g Fe^{2+})
Distilled Water	Rind	1.95 ± 0.05 ^{a}	3.00 ± 0.05 ^{a}	60.18 ± 0.74 ^{a}	19.61 ± 0.16 ^{a}	42.75 ± 2.26 ^{a}
	Flesh	0.35 ± 0.03 ^{a}	0.68 ± 0.09 ^{b}	6.43 ± 0.20 ^{b}	4.32 ± 0.47 ^{b}	5.86 ± 0.41 ^{b}
	Kernel	1.03 ± 0.32 ^{a}	1.52 ± 0.31 ^{c}	5.75 ± 0.20 ^{b}	11.36 ± 0.66 ^{c}	7.27 ± 0.07 ^{b}
Methanol	Rind	40.75 ± 1.45 ^{b}	10.31 ± 0.12 ^{d}	75.72 ± 0.17 ^{c}	20.65 ± 0.09 ^{a}	95.17 ± 0.41 ^{c}
	Flesh	3.09 ± 0.14 ^{a}	1.54 ± 0.06 ^{c}	34.50 ± 2.71 ^{d}	12.90 ± 1.09 ^{c}	15.01 ± 0.65 ^{d}
	Kernel	6.49 ± 0.04 ^{a}	1.57 ± 0.02 ^{c}	22.24 ± 4.16 ^{e}	11.82 ± 0.44 ^{c}	12.49 ± 0.60 ^{e}
Ethanol	Rind	68.98 ± 6.71 ^{c}	10.62 ± 0.11 ^{d}	74.54 ± 0.87 ^{c}	19.01 ± 0.13 ^{a}	110.20 ± 0.03 ^{f}
	Flesh	1.38 ± 0.07 ^{a}	0.88 ± 0.07 ^{b}	26.69 ± 2.70 ^{e}	8.87 ± 1.61 ^{d}	11.28 ± 0.23 ^{e}
	Kernel	2.26 ± 0.21 ^{a}	1.56 ± 0.06 ^{c}	15.12 ± 1.33 ^{f}	11.50 ± 0.67 ^{c}	12.00 ± 0.28 ^{e}

Results were presented as mean ± standard deviation of triplicates (n = 3). Different letters within the same column indicate a significant difference at $p < 0.05$.

Total phenolic content (TPC) was between 0.68 ± 0.09 mg GAE/g (water extract of flesh) and 10.62 ± 0.11 mg GAE/g (ethanolic extract of rind). All rind extracts exhibited significantly higher ($p < 0.05$) TPC than flesh and kernel extracts, even though extraction was carried out using three different pure solvents. The TPC content of rind was in the ascending order of distilled water extract (3.00 ± 0.05 mg GAE/g) < methanolic extract (10.31 ± 0.12 mg GAE/g) < ethanolic extract (10.62 ± 0.11 mg GAE/g), indicating that organic solvents were still a better choice in extracting phenolic compounds of *pulasan*. These findings are generally consistent with those previously reported by Tan et al. [8], Sopee et al. [17], and Do et al. [18]. A previous study by Sopee et al. [17] showed that the TPC of *pulasan* rind was 1.07 mg GAE/g (water extract) and 0.92 to 1.00 mg GAE/g (40–80% ethanolic extracts), respectively. Interestingly, the TPC of the rind extracts in the current study was 2-fold (water extract) and 10-fold higher (ethanolic extract), compared to the findings of Sopee et al. [17].

3.2. Quantification of Antioxidant Capacity in Different Parts of Pulasan

3.2.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH radical scavenging assay is intended to measure the ability of bioactive compounds in scavenging the DPPH radicals. The discoloration of the deep violet DPPH reagent to colorless or yellow indicates the presence of bioactive compounds [19]. The DPPH radical scavenging activity was in between 5.75 ± 0.20% RSA (water extract of the kernel) and 75.72 ± 0.17% RSA (methanolic extract of rind) (Table 1). Similar to the previously reported TFC and TPC, *pulasan* rind had a significantly higher ($p < 0.05$) DPPH radical scavenging activity than that of flesh and kernel in all extraction solvents. Another finding worth highlighting is that organic solvents (methanol and ethanol) demonstrated a higher DPPH radical scavenging activity than distilled water in all parts of *pulasan*. In general, there was only a marginal difference in the DPPH radical scavenging of ethanolic and methanolic extracts of all sample extracts. These findings are broadly in-line with a previous study by Iloki-Assanga et al. [20], who reported that a highly polar solvent (such as water) is less effective in extracting bioactive compounds. It is also worth mentioning that the DPPH radical scavenging activities of *pulasan* rind in the current study were slightly lower than water extracts (80.4%) and 40–80% ethanolic extracts (58.0–88.9%) of those previously reported in the literature [17].

3.2.2. Trolox Equivalent Antioxidant Capacity (TEAC)

The TEAC assay quantifies the bleaching capability of bioactive compounds against the $ABTS^{+}$ radical cations [21]. The TEAC values of all sample extracts were in the range of 4.32 ± 0.47 μmol/g T.E. (water extract of flesh) to 20.65 ± 0.09 μmol/g T.E. (methanolic extract of rind) (Table 1). The rind showed the highest TEAC values in all three pure solvents (methanol, 20.65 ± 0.09 μmol/g T.E.; distilled water, 19.61 ± 0.16 μmol/g T.E.;

ethanol, 19.01 ± 0.13 μmol/g T.E.) as compared to fruit and kernel. The methanolic extract of rind had a slightly higher TEAC value than distilled water and pure ethanol extracts. In short, these findings suggest that all three extraction solvents had a comparable $ABTS^+$ reducing ability.

3.2.3. Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay quantifies the capability of bioactive compounds in reducing the Fe^{3+}-tripyridyltriazine to ferrous (Fe^{2+}) complex under an acidic (pH 3.6) condition [21]. The FRAP values were in the range of 5.86 ± 0.41 μmol/g Fe^{2+} (water extract of flesh) to 110.20 ± 0.03 μmol/g Fe^{2+} (ethanolic extract of rind) (Table 1). Of the three parts investigated, the FRAP value of the rind was significantly higher ($p < 0.05$) than the fruit and kernel, regardless of the extraction solvent. It is also worth highlighting that the FRAP values of organic solvents were approximately 2-fold higher than those reported in water extracts. These findings were generally in contradiction to the findings of Sopee et al. [17], who reported that the water extract of *pulasan* rind had a higher FRAP value as opposed to the 40–80% ethanolic extracts.

3.3. The Anti-Hypertensive Properties of Pulasan Rind

It is obvious that the *pulasan* rind is a potent antioxidant source compared to its flesh and kernel. Therefore, the anti-hypertensive and hypoglycemic properties of the *pulasan* rind were further assessed with the angiotensin-converting enzyme (ACE) inhibitory assay and alpha-amylase inhibitory assay, respectively. The hippuryl-L-histidyl-L-leucine (HHL) serves as a substrate for ACE, in which it is hydrolyzed to hippuric acid (H.A.) in the presence of anti-hypertensive compounds. Therefore, the concentration of H.A. is directly proportionate to the anti-hypertensive property of a sample extract [22]. Table 2 depicts the ACE inhibition activity of *pulasan* rind in different extraction solvents. Findings in the current study indicated that water extract had the lowest ACE inhibition activity (83.97% ± 2.79%), followed by the methanolic extract (97.74% ± 0.72%) and ethanolic extract (98.93% ± 0.58%). The high ACE inhibitory activities suggested that the rind of the *pulasan* is a potential source of the anti-hypertensive agent. A recent study by Tan et al. [8] highlighted that the ACE inhibitory activity of *salak* peel was 86.94%−98.96%. By comparison, the ACE inhibitory activity of *pulasan* rind was comparable to that reported in the *salak* peel.

Table 2. The anti-hypertensive and hypoglycemic properties of *pulasan* rind in various extraction solvents.

Extraction Solvent	Rind
ACE inhibition (%)	
Distilled Water	83.97 ± 2.79 [a]
Methanol	97.74 ± 0.72 [b]
Ethanol	98.93 ± 0.58 [b]
Alpha-amylase inhibition (%)	
Distilled Water	83.84 ± 2.34 [c]
Methanol	96.16 ± 10.23 [d]
Ethanol	94.89 ± 6.39 [d]

Results were presented as mean ± standard deviation of triplicates ($n = 3$). Different letters within the same column indicate a significant difference at $p < 0.05$.

3.4. The Hypoglycemic Properties of Pulasan Rind

The current study also examined the hypoglycemic properties of the *pulasan* rind through the alpha-amylase inhibitory assay. Of the three extraction solvents, the methanolic extract (96.16 ± 10.23%) exhibited a slightly higher inhibitory activity than water extract (83.84 ± 2.34%) and ethanolic extract (94.89 ± 6.39%) (Table 2). This contradicts with the findings reported by Kamtekar et al. [23], who suggested that aqueous extract has better

inhibitory activity than ethanol. In general, the rind of *pulasan* had alpha-amylase inhibitory potential irrespective of the extraction solvents. However, the current study acknowledged that extraction conditions (duration and temperature) are among the factors determining the concentration of the extracted bioactive compounds [12]. This may significantly impact the reported anti-hypertensive and hypoglycemic effects of the rind extract. Therefore, a future study may consider the impacts of the extraction conditions of the bioactive compounds on the anti-hypertensive and hypoglycemic properties.

3.5. Bioactive Compounds of Pulasan Rind

Organic solvents (methanol and ethanol) have been shown to possess higher antioxidant capacity, anti-hypertensive and hypoglycemic effects than distilled water. Therefore, bioactive compounds in *pulasan* rind were identified using HPLC with ethanol as the extraction solvent. Of the two organic solvents, ethanol was selected because it is more practical to be used in the development of nutraceuticals, owing to its non-toxic characteristic [16]. Geraniin, chlorogenic acid, catechin, corilagin, syringic acid, ellagic acid, and naringenin were among the bioactive compounds identified from the ethanol extracted from *pulasan* rind (Table 3). It is obvious that chlorogenic acid was the main bioactive compound of *pulasan* rind (107.10 ± 1.09 mg/g), followed by geraniin (77.09 ± 0.63 mg/g), corilagin (41.93 ± 0.28 mg/g), ellagic acid (35.12 ± 2.27 mg/g), naringenin (23.53 ± 0.95 mg/g), catechin (21.30 ± 0.80 mg/g) and lastly syringic acid (6.95 ± 0.42 mg/g). Findings in the current study are broadly in line with a previous study by Thitilertdecha et al. [3], indicating that ellagic acid, corilagin, and geraniin are the main bioactive compounds of *rambutan*.

Table 3. The bioactive compounds composition of ethanolic extract of *pulasan* rind.

Bioactive Compounds	mg/g Sample
Geraniin	77.09 ± 0.63
Chlorogenic acid	107.10 ± 1.09
Catechin	21.30 ± 0.80
Corilagin	41.93 ± 0.28
Syringic acid	6.95 ± 0.42
Ellagic acid	35.12 ± 2.27
Naringenin	23.53 ± 0.95

Results were expressed as mean ± standard deviation of triplicate samples (n = 3).

3.6. Identification of the Anti-Hypertensive and Hypoglycemic Bioactive Compounds Derived from the Pulasan Rind

Table 4 shows the correlations between the antioxidant properties, bioactive compound, anti-hypertensive and hypoglycemic properties of *pulasan* rind. Positive and significant correlations ($p < 0.05$) were observed in TPC and ACE inhibitory activity (r = 0.977), TFC and ACE inhibitory activity (r = 0.913), FRAP and ACE inhibitory activity (r = 0.976) as well as DPPH and ACE inhibitory activity (r = 0.971). Further analysis revealed that geraniin (r = 0.998), chlorogenic acid (r = 0.997), catechin (r = 0.998), corilagin (r = 0.997), syringic acid (r = 0.998) and naringenin (r = 0.998) were positively correlated with the ACE inhibitory activity of *pulasan* rind. Overall, these findings support the notion that the anti-hypertensive properties of *pulasan* rind were attributed to those previously mentioned bioactive compounds.

On the other hand, positive and significant correlations ($p < 0.05$) were noted in TPC and alpha-amylase inhibitory activity (r = 0.691) as well as DPPH and alpha-amylase inhibitory activity (r = 0.707). However, no significant correlation was observed between individual bioactive compounds and the alpha-amylase inhibitory activity of *pulasan* rind. Future studies may profile the potential phenolics that contribute to the hypoglycemic properties of *pulasan* rind.

Table 4. Correlations between the studied variables.

Antioxidant Properties	ACE Inhibition	Alpha-Amylase Inhibition
TPC	0.977 *	0.691 *
TFC	0.913 *	0.633
FRAP	0.976 *	0.653
TEAC	0.061	0.143
DPPH	0.971 *	0.707 *
Bioactive Compound	**ACE Inhibition**	**Alpha-Amylase Inhibition**
Geraniin	0.998 *	0.845
Chlorogenic Acid	0.997 *	0.846
Catechin	0.998 *	0.845
Corilagin	0.997 *	0.846
Syringic Acid	0.998 *	0.845
Ellagic Acid	−0.998	−0.845
Naringenin	0.998 *	0.845

*: Correlation is significant at the 0.05 level (2-tailed).

4. Conclusions

Findings in the current study demonstrated that *pulasan* rind and the other by-products of *pulasan* possess a significant antioxidant capacity. Moreover, geraniin, chlorogenic acid, catechin, corilagin, syringic acid, and naringenin of *pulasan* rind may function as anti-hypertensive agents. Further investigations using solvents at different polarities could be explored to obtain a more comprehensive picture of the bioactive compounds present in the *pulasan* rind. Since findings in the current study suggested a promising effect of bioactive compounds against hypertension and glycemic control, in vivo study involving animal models can be executed in the future to confirm the possible mechanism of actions.

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