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Storage Effect on Phenolic Compounds and Antioxidant Activity of *Nephelium lappaceum* L. Extract

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Abstract: Preparation of potential antioxidant extracts with less process for storing in a long period is preferable. *N. lappaceum* rind, well known as a promising source of phenolic antioxidants agricultural residue, was employed to prepare crude extracts by different solvents. The phenolic content, flavonoid content, antioxidant, and anti-tyrosinase activities of the extracts were evaluated. The stability of the potential extract was then assessed for phenolic content and antioxidant activity under various storage conditions. The extractive yields of crude phenolic extract ranged from 16.61 to 28.78%. The ethanolic extract of *N. lappaceum* rind exhibited potential antioxidant activities and contained a high amount of phenolics and flavonoid contents. The extract remained with a high amount of the phenolic content (up to 88.79%) and retained its antioxidant property under various temperatures (4, 25, and 45 °C) after the first week of the storage period. The results suggest that phenolic content and antioxidant activity of *N. lappaceum* rind extract, as a nutraceutical or anti-aging ingredients in cosmetics, could be stored at a temperature from 4 °C to 45 °C with or without oxygen exposure at least for 16 weeks.

Keywords: antioxidant; *Nephelium lappaceum* L.; phenolic compounds; stability; tyrosinase inhibition

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1. Introduction

Reactive oxygen species (ROS) or free radicals generated from internal living organisms could function in many biological pathways in the human body. However, an excess amount of these free radicals cause oxidative stress. When accumulated over a long period, they could lead to deterioration in functions of internal organs as well as aging and pigmentation in skin [1]. In order to extend cell life span, the promotion of antioxidant systems and/or antioxidant supplementation is considered as interesting approaches. Plants contain primary and secondary metabolites, and the latter can be classified into various types including terpenes, saponins, alkaloids, glycosides, phenolics, etc. These have been revealed for their biological properties, such as anti-microbial [2], anti-inflammatory [3], anti-diabetic [4], anti-hyperglycemic [5], as well as antioxidant properties [6]. Phenolic compounds are the most abundant metabolites occurring in nature and most of them are attributed to antioxidant properties, due to their structure containing hydroxyl group bonded with aromatic ring [7]. *Nephelium lappaceum* L. (rambutan), a tropical fruit extensively grown in South-East Asia, is commonly processed in the food industry and its non-edible portions are normally discarded. However, these residuals have been recognized as feasible for increasing the value for the utilization as nutraceutical, medicine, and industries in the past decade. Rambutan rind has been found to be one of the alternative sources of phenolic antioxidants, particularly geraniin and other minor components including corilagin, ellagic acid, and gallic acid [8]. Its rind exhibits various biological functions which could be applied to nutraceutical and pharmacological purposes. These include anti-microbial [6], anti-inflammatory [9], anti-glycemic [5], anti-diabetic [4], anti-tyrosinase [10], and antioxidant properties [6], which are responsible for broad range of biological functions. Similarly,

various types of solvents, e.g., methanol, water, and ethyl acetate fraction from 70% ethanol, are used in the extraction influences on the number of phytochemical compounds and biological potential [6,10,11]. In the application of herbal extracts in cosmetics and pharmaceutical industries, the stability of the extract is also a concern due to the degradation of the phytochemicals that cause an unpreferable impact on the quality of the extracts. Through stability investigation, various environmental conditions are used to assess the quality of the extracts in finding proper storage conditions. Although Phenolic compounds from rambutan rind are preferably applied as antioxidants and other bioactive properties, however, no study on the stability of its phenolic content and antioxidant property has been previously published. Therefore, various rambutan rind crude extracts were evaluated for phenolic and flavonoid content, antioxidant, and anti-tyrosinase activities for the selected extract. The selected crude extract was then investigated for the stability of phenolic content and antioxidant property under various storage conditions for 16 weeks.

2. Materials and Methods

2.1. Materials and Chemicals

Rambutan fruit (Rongrien cultivar) was collected from the Chanthaburi province of Thailand in July 2015. Chemicals of analytical grade, including 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), Folin–Ciocalteu's reagent, gallic acid, ascorbic acid, quercetin, kojic acid, tyrosinase, L-3,4-dihydroxyphenylalanine (L-DOPA), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2,4,6-tris (2-pyridyl)-*s*-triazine (TPTZ), and others were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of Crude Phenolic Extracts from Nephelium lappaceum Rind

The rambutan fruit was separated and its rind was rinsed with distilled water. To compare the effect of different drying processes on total phenolic content and $DPPH^{\bullet}$ scavenging activity, the sample was separately dried by ventilating in a hot air oven at 50 °C, freeze dried, shade dried, and extracted by using ethanol with the condition described below. Ventilating in a hot air oven had been found to provide the highest amounts of both phenolic content and $DPPH^{\bullet}$ scavenging activity (137.00 ± 6.54 mg GAE/g sample) indicating ventilating hot air oven at 50 °C was suitable in the drying process. After the rambutan rind was dried, it was powdered and sieved through 250-mesh. The obtained sample powder was then used to prepare crude phenolic extracts. Ethanol, as an extraction solvent, had more potential than methanol to recover phenolic compounds from the rambutan rind [12] and was regarded as safe. Therefore, in this study, ethanol, 75% (*v*/*v*) ethanol, and water were used for the preparation of crude extracts through the solid–liquid extraction method. Briefly, the sample (10 g) suspended in different solvents with an LS ratio of 15:1 (mL:g) was shaken with an orbital shaker (150 rpm) at ambient temperature for 12 h. The extract was filtered through Whatman® filter paper no.1, evaporated, and lyophilized. The obtained crude phenolic extracts were stored at −20 °C until analysis.

2.3. Storage Conditions

The storage conditions were conducted according to the methods modified from Del-Toro-Sánchez et al. [13]. The powder of phenolic crude extracts was transferred into a light-protected glass bottle. The solution form in distilled water was prepared and stored at 25 °C. The samples were stored at 4 °C, 25 °C, and 45 °C in a closed lid to determine the extract's stability. The oxygen exposure in the opened bottle at 25 °C and 45 °C was also prepared to evaluate the influence of oxygen on the stability of phenolic content and on the $DPPH^{\bullet}$ scavenging activity.

2.4. Determination of the Total Phenolic Content

The total phenolic content was estimated according to Folin–Ciocalteu's assay [14]. Samples (1 mL) were mixed with deionized water (7 mL). After that, Folin–Ciocalteu's reagent (0.5 mL) was added, followed by 200 g/L of sodium carbonate solution (1.5 mL).

The absorbance of 760 nm was measured after the mixtures were shaken and incubated at an ambient temperature for 1 h. The results were expressed as milligrams of gallic acid equivalents, per gram of extract (mg GAE/g extract).

2.5. Determination of the Total Flavonoid Content

The total flavonoid content was estimated based on an aluminum chelating assay according to the method described by Prommuak et al. [15] with slight modifications. Samples (1 mL) were mixed with 100 g/L of an aluminum chloride solution (0.1 mL), followed by 1 M of potassium acetate solution (0.1 mL). The mixture was then immediately made up to 5 mL with water. The absorbance of 415 nm was measured after the mixtures were shaken and incubated at an ambient temperature for 30 min. The results were expressed as milligrams of quercetin equivalents, per gram of extract (mg QE/g extract).

2.6. Antioxidant Assays

2.6.1. DPPH$^{\bullet}$ Scavenging Assay

The scavenging activity of 2,2-diphenyl-1-picryl-hydracyl (DPPH$^{\bullet}$) was evaluated based on the method described by Gülçin et al. [16]. A 0.1 mM DPPH$^{\bullet}$ solution in ethanol (3 mL) was mixed with 1 mL of sample solution. A control, comprising 1 mL of ethanol and 3 mL of DPPH$^{\bullet}$ solution, was prepared. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer (SP-880, Metertech, Taipei, Taiwan) after the mixture was incubated at ambient temperature in dark conditions for 30 min. The DPPH$^{\bullet}$ scavenging activity was calculated according to the following equation:

$$\text{Antioxidant activity (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the mixture with the sample. The IC_{50} of reference antioxidant compounds, gallic acid, and ascorbic acid were used as the comparative standard to the IC_{50} value of the extracts.

2.6.2. ABTS$^{\bullet+}$ Scavenging Assay

The scavenging activity of the monocation radical of ABTS (ABTS$^{\bullet+}$) was evaluated based on the method previously described by Re et al. [17]. The ABTS$^{\bullet+}$ was generated by the oxidation of ABTS with potassium persulfate. An aliquot (0.1 mL) of each sample was mixed with 0.9 mL of 20 mM phosphate buffer (pH 7.4). After that, ABTS$^{\bullet+}$ reagent (2.0 mL) was added to the mixture and incubated at an ambient temperature in dark conditions for 30 min. A control, comprising 1.0 mL of 20 mM phosphate buffer (pH 7.4) and ABTS$^{\bullet+}$ reagent (2.0 mL), was prepared. The absorbance was measured at 734 nm using a UV-Vis spectrophotometer. The ABTS$^{\bullet+}$ scavenging activity was calculated as the equation mentioned above. IC_{50} of reference antioxidant compounds, gallic acid, and ascorbic acid were used as the comparative standard to IC_{50} value of the extracts.

2.7. Tyrosinase Inhibitory Assay

The inhibition of the tyrosinase enzyme was evaluated based on the dopachrome method as previously described by Mendes et al. [18]. An aliquot (20 µL) of each sample was diluted with 110 µL of 50 mM phosphate buffer (pH 6.8) and then mixed with 20 µL of a mushroom tyrosinase solution (1000 units/mL). After the mixture was pre-incubated at 37 °C for 10 min, 50 µL of L-DOPA solution (10 mM) was added to initiate an enzymatic reaction. The absorbance was measured at 475 nm after the reaction was incubated at 37 °C for 10 min. The control, containing 130 µL of 50 mM phosphate buffer (pH 6.8), 20 µL of tyrosinase solution (1000 units/mL), and 50 µL of 10 mM L-DOPA was prepared. The IC_{50} of reference anti-tyrosinase compounds, kojic acid, and ellagic acid were used for comparison to IC_{50} of the extracts.

2.8. Statistical Analysis

All measurements were performed in triplicate and the results were expressed as mean ± S.D. The results were statistically analyzed via one-way analysis of variance (ANOVA) through Duncan's multiple range test using SPSS Program version 21.0 (SPSS Inc., Chicago, IL, USA). The statistical differences were considered as significant at $p < 0.05$.

3. Results and Discussions

3.1. Extraction Yield of N. lappaceum Crude Extracts

The solid–liquid extraction technique is a well-known conventional extraction process extensively used to extract natural bioactive compounds from natural sources. Energy-assisted extraction methods have been used to improve phenolic extraction efficiency. Phuong et al. (2020) employed ultrasound-assisted extraction for extracting phenolic to enhance extraction efficiency by around 15% [19]. Thermal factor is one of the necessary concerns for the heat-sensitive compounds degraded in the extraction process with high temperature. The temperature not over 50 °C seemed to cause less degradation of phenolic compounds during the extraction process [19,20]. Therefore, the solid-liquid extraction technique was employed based on the shaking method at ambient temperature. To study the effect of solvent on the amount of phenolic and flavonoid contents, antioxidant activities and tyrosinase inhibitory activity of rambutan rind, its powder was extracted using different solvents, including water, 75% (*v*/*v*) ethanol, and ethanol. After extraction, the 75% (*v*/*v*) ethanolic extract was found to be the highest extractive yield of 28.78% (*w*/*w*) followed by water extract (18.04%), and ethanolic extract (16.61%) (Table 1), indicating that the solvents had a significant influence on the extractive yield. Polarity and solubility play an important role in extracting phenolic compounds. The single solvent used in this study provided a lower extractive yield compared with the solvent mixture (75% (*v*/*v*) ethanol). These findings might be explained that the bioactive compounds in rambutan rind could be suitable to be solubilized in 75% (*v*/*v*) ethanol. The obtained extracts were then estimated for phenolic and flavonoid contents, evaluated for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities and tyrosinase inhibitory activity.

Table 1. Extractive yield, phenolic content, flavonoid content, and biological properties of *Nephelium lappaceum* extracts.

Scheme 50	Extractive Yield (%)	Total Phenolic Content (mg GAE/g Extract)	Total Flavonoid Content (mg QE/g Extract)	DPPH Scavenging IC_{50} (μg/mL)	ABTS Scavenging IC_{50} (μg/mL)	Tyrosinase Inhibition IC_{50} (μg/mL)
Water	18.04 ± 0.74^{b}	219.49 ± 4.41^{c}	13.93 ± 0.31^{b}	28.03 ± 0.60^{b}	29.31 ± 0.61^{b}	$>1000^{b}$
75% (*v*/*v*) ethanol	28.78 ± 0.88^{a}	353.77 ± 2.84^{b}	11.95 ± 0.24^{c}	47.34 ± 0.19^{c}	32.09 ± 3.45^{b}	$>1000^{b}$
Ethanol	16.61 ± 1.56^{b}	483.72 ± 32.95^{a}	23.37 ± 0.46^{a}	8.72 ± 0.27^{a}	19.20 ± 0.18^{a}	$>1000^{b}$
Gallic acid	-	-	-	49.52 ± 0.05^{d}	56.24 ± 0.18^{c}	-
Ascorbic acid	-	-	-	111.70 ± 0.23^{e}	103.31 ± 0.25^{d}	-
Kojic acid	-	-	-	-	-	150.00 ± 30.64^{a}
Ellagic acid	-	-	-	-	-	192.39 ± 40.73^{a}

Note: Lowercase superscripts mean the column followed by different letters are significantly different ($p < 0.05$).

3.2. Determination of Phenolic and Flavonoid Contents

Rambutan is well known as a promising source of phenolic compounds, including ellagitannins and their derivatives. Recently, rambutan rind has been shown to contain many flavonoids. These compounds exhibit various bioactivities with an essential function in pharmacological and medicinal approaches. Generally, a higher amount of phenolic and flavonoid compounds is preferable according to the standardization of the plant extracts. The extract with the highest amount of phenolic and flavonoid was one consideration criteria for choosing to be assessed for its stability under various storage conditions.

The contents of phenolic compounds in different rambutan rind extracted using different solvents are shown in Table 1. This indicated that the solvent had a significant influence on the extraction of phenolic compounds in rambutan rind. The chemical structure of phe-

nolic compounds varied from simple to complex structures, resulting in various solubility properties in different extraction solvents [21]. The highest amount of phenolic content was obtained by using ethanol (483.72 ± 3 2.95 mg GAE/g extract), in contrast to rambutan rind extracted by water. It showed that the least phenolic content was lower than half of the ethanolic extract. Alcoholic solvents such as methanol and ethanol had more potential to extract phenolic compounds than water [12,19].

As rambutan rind contain flavonoids as chemical constituents [11], flavonoid content of rambutan rind extracted was also estimated. Flavonoids are the largest group of phenolic compounds and are the most abundant in plants. As shown in Table 1, all extracts that contained flavonoids as chemical constituents ranged from 13.93 ± 0.31 to 23.37 ± 0.46 mg QE/g extract. Similar to those of phenolic content, these possessed the highest flavonoid content was obtained by using ethanol ($p < 0.05$). Previous studies reveal that geraniin is the major constituent in rambutan rind [5,8]. Therefore, geraniin in the ethanolic extract was identified and quantified for its amount in the ethanolic extract by using UPLC. As reported in previous work, its geraniin content was 474.9 mg/g extract [22]. This study is inconsistent with previous studies that found flavonoid content with higher ratio to phenolic content (~28% [23], ~23% [24], ~13% [25])—this study was (~5%). Nevertheless, phenolic content in this study was twice that when compared with the study of [23] indicating that the content of bioactive compounds depended on not only plant species but also growing location. Considering phenolic and flavonoid content, ethanol is suitable to be used as an extraction solvent for the phenolic extraction from rambutan rind.

3.3. Antioxidant Activities

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated at cellular level and in various external environmental conditions, including UV rays, chemicals, and air pollution, are recognized to be highly reactive molecules. They damage DNA, proteins, and lipids, resulting in deterioration of the skin and health. This will finally lead to the formation of aging and many degenerative diseases. Antioxidants are well known as compounds that play important roles to inhibit free radical formations and/or to neutralize free radicals. The latter has been extensively employed to evaluate antioxidant properties of natural sources.

The *N. lappaceum* extracts were evaluated for their capability to scavenge DPPH$^{\bullet}$ and ABTS$^{\bullet+}$. The results are shown in Table 2. The IC_{50} value of DPPH$^{\bullet}$ scavenging potential ranged between 8.72 and 47.34 µg/mL. The most potential to scavenge DPPH$^{\bullet}$ was the rambutan rind extracted by using ethanol, followed by the water, and the 75% (*v*/*v*) ethanol. Interestingly, all crude extracts were more potential to scavenge DPPH$^{\bullet}$ when compared to gallic acid (49.52 µg/mL) and ascorbic acid (111.70 µg/mL) ($p < 0.05$). This result was in agreement with previous studies [6,12]. The monocation radical scavenging activity through ABTS assay was also evaluated and expressed as IC_{50} values. The results were similar to those of DPPH$^{\bullet}$ scavenging results that rambutan rind extracted by ethanol was the most effective to scavenge monocation radical ($p < 0.05$). Water and 75% (*v*/*v*) ethanol as extraction solvent provided the extracts with no difference in scavenging monocation ($p > 0.05$). Considering the comparative antioxidants, rambutan rind extracts were more significantly effective ($p < 0.05$). Correlations among the phenolic and flavonoid contents and antioxidant activities were also determined. There was substantial correlation between the flavonoid contents versus DPPH$^{\bullet}$ ($R^2 = -0.934$) and ABTS$^{\bullet+}$ ($R^2 = -0.956$) scavenging activities as well as phenolic content ($R^2 = 0.762$). The phenolic content also correlated with ABTS$^{\bullet+}$ scavenging activity ($R^2 = -0.707$) while a moderate relationship was observed with DPPH$^{\bullet}$ scavenging activity ($R^2 = -0.486$). The chemical structure of phenolic compounds has been revealed as has the number and position of the active hydroxyl group (-OH) influence on the DPPH$^{\bullet}$ scavenging property [7]. Different profiles of phenolic compounds had a significant influence on various types of antioxidant properties [12]. These findings indicate that ethanol has been proven suitable for the extraction of phenolic antioxidants from rambutan rind.

Table 2. Stability in total phenolic compounds of the ethanolic extract under various conditions.

Week	Total Phenolic Content (mg GAE/g Extract)					
	4 °C Closed	**25 °C Closed**	**25 °C Opened**	**45 °C Closed**	**45 °C Opened**	**Solution**
0	394.97 ± 32.83 ^{a,A}	394.97 ± 32.83 ^{a,A}	394.97 ± 32.83 ^{a,A}	394.97 ± 32.83 ^{a,A}	394.97 ± 32.83 ^{a,A}	394.97 ± 32.83 ^{a,A}
1	376.00 ± 15.12 ^{a,b,A}	389.22 ± 12.28 ^{a,b,A}	390.76 ± 11.95 ^{a,A}	371.04 ± 26.19 ^{a,A}	391.279 ± 11.32 ^{a,b,A}	390.03 ± 10.57 ^{a,b,A}
2	388.87 ± 29.57 ^{a,b,A}	391.04 ± 32.02 ^{a,A}	375.07 ± 23.24 ^{a,A}	402.12 ± 32.52 ^{a,A}	390.99 ± 31.57 ^{a,b,A}	397.36 ± 40.82 ^{a,A}
4	360.53 ± 20.05 ^{a,b,B,C,D}	371.86 ± 13.90 ^{a,b,A,B,C}	356.66 ± 6.11 ^{a,C,D}	377.23 ± 9.71 ^{a,A,B}	380.40 ± 11.93 ^{a,b,A}	342.83 ± 4.9 ^{a,b,D}
6	358.26 ± 22.22 ^{a,b,A,B}	356.27 ± 1.95 ^{a,b,A,B}	N.D.	363.64 ± 3.89 ^{a,A,B}	387.54 ± 39.90 ^{a,b,A}	345.00 ± 2.73 ^{a,b,B}
10	361.48 ± 14.24 ^{a,b,A}	347.44 ± 6.20 ^{a,b,A,B}	N.D.	353.22 ± 9.97 ^{a,A}	363.08 ± 12.30 ^{a,b,A}	334.78 ± 6.43 ^{b,B}
16	335.31 ± 11.70 ^{b,A}	336.02 ± 10.41 ^{b,A}	N.D.	350.70 ± 6.59 ^{a,A}	333.47 ± 9.07 ^{b,A}	339.99 ± 40.68 ^{a,b,A}

Note: Lowercase superscripts mean the column followed by different letters are significantly different ($p < 0.05$). Uppercase superscripts mean the row followed by different letters are significantly different ($p < 0.05$). N.D.; not determined.

3.4. Anti-Tyrosinase Activity

The inhibition of tyrosinase is one of the mechanisms which inhibit melanogenesis in skin. Thus, the extracts were evaluated for their capability to inhibit tyrosinase compared with similar anti-tyrosinase agents, kojic acid and ellagic acid. As shown in Table 1, all extracts revealed less effectiveness on the inhibition of tyrosinase enzyme (>1000 μg/mL) when compared with kojic acid and ellagic acid ($p < 0.05$). The rambutan rind contains geraniin as the major phenolic constituent along with minor phenolics including ellagic acid and gallic acid. Ellagic acid is considered a lightening agent that not only interrupts the melanogenesis process in the oxidation of L-tyrosine and/or L-DOPA catalyzed by the tyrosinase enzyme but also reduces the o-dopaquinone. The o-diphenol structure in the ellagic acid molecule could function as a substrate competitor with L-tyrosine and L-DOPA [26]. Although geraniin has one ellagic acid in its molecule, the steric hindrance effect of geraniin might interrupt the process for binding at the active site of the tyrosinase enzyme.

For the selection of the rambutan rind extract to be further evaluated for its stability, the rambutan extracted by ethanol is emphasized to be chosen according to its high contents of phenolics and flavonoids, incorporated with a strong antioxidant properties.

3.5. Stabilities of Phenolic Content and Antioxidant Activity under Storage Condition

The stability of phenolic compounds under various storage conditions plays an important factor to ensure the extract quality. Temperature, water, oxygen, and solubilized form are important factors that could influence the phenolics' stability. Therefore, the phenolic content and antioxidant activity stabilities of the ethanolic extract of rambutan rind were evaluated under various conditions including temperature (4 °C, 25 °C, and 45 °C), oxygen exposure (closed and opened container at 25 and 45 °C), as well as an aqueous solution (25 °C). The total phenolic content changes in the ethanolic extract with different temperature, exposure to oxygen, and the solution formed during storage are shown in Table 2. Unfortunately, the extract exposed to oxygen at 25 °C was not complete for 16 weeks because the extracts became sticky from moisture absorption at the sixth week. The total phenolic decreased at all range temperatures for 16 weeks ($p < 0.05$) except at 45 °C, which slightly decreased with no significance ($p > 0.05$). However, it was interesting that the total phenolic content of the extract stored under all storage conditions was not statistically different ($p > 0.05$).

Overall, the remaining total phenolic contents of the ethanolic extract under all storage conditions were higher than 80% and ranged from 84.42 to 88.79% at the 16th week. Many studies focus on the stability of the phenolic compounds in the extracts due to the quality of the functional active ingredients in the extract. The phenolic compounds of the Cornelian cherry were evaluated for their half-life value for 142.01 and 17.83 days under a temperature of 22 °C and 55 °C, respectively [27]. The rambutan rind phenolics has an estimated half-life value longer than 112 days at all condition indicating the stability potential of rambutan rind phenolics. Different types of phenolic compounds vary in their stability. For example, phenolic compounds of *Anemopsis californica* have half-life values longer than 180 days

under light exposure at 25 °C [13] and anthocyanins have half-life values up to 17 weeks. However, light protection and lower temperature are required [28]. Physical factors, such as oxygen exposure and temperature, are important factors for the degradation of phenolic compounds [29].

The geraniin, a major constituent of *N. lappaceum* rind, can be hydrolyzed or decomposed to yield brevifolin carboxylic acid and corilagin, which the latter could also be hydrolyzed to obtain ellagic acid and gallic acid [30]. The formation of brevifolin carboxylic acid from hexahydroxydiphenoyl (HHDP) of geraniin is the oxidative reaction [31]. In addition, hexahydroxybiphthalic acid, galloyl-bis-HHDP-glucose, and brevifolin are also degradation products of geraniin [32]. High temperatures at 60 °C and 100 °C for 10 h dramatically cause geraniin degradation of 17.13% and 100%, respectively. In addition, lower temperature at 40 °C can also lead to the degradation of 18.13% when compared with the deep freeze condition [32]. These might be explained, in this study, for the gradual decrement of phenolic contents at all storage conditions. These findings indicate that temperature ranges from 4 °C to 45 °C with humidity control could maintain the phenolic compounds in the rambutan rind extract.

The antioxidant stability of the rambutan rind ethanolic extract was also evaluated under similar conditions to those of phenolic stability; the results are shown in Table 3. Antioxidant activity dramatically decreased at all storage conditions in the first week. However, at the end of the study, there was no significant change in antioxidant activity in all storage conditions, indicating that the decrement of phenolic content along the storage period had less effect on antioxidant property, especially after the first week. Similarly, the antioxidant activity of the ethanolic extract under various conditions at the end of the study was not statistically different ($p > 0.05$). The oxidative process could breakdown geraniin into many degradation products, as mentioned above. This phenomenon might affect the antioxidant property of the extract because each phenolic compound exhibits a different level of antioxidant potential. The ability of the geraniin and its degradation products to scavenge DPPH is ranked as: geraniin >> corilagin > ellagic acid >> gallic acid >> brevifolin carboxylic acid [8,33]. The degradation of geraniin yielding corilagin and brevifolin carboxylic acid, especially at the first week, may dramatically reduce the overall antioxidant property of the extract in all conditions. The degradation profile of the extract under various storage conditions should be further studied to explain the profile of the degradation pathway.

Table 3. Stability in antioxidant activity of the ethanolic extract under various conditions.

Weeks	IC_{50} (μg/mL)					
	4 °C	**25 °C Closed**	**25 °C Opened**	**45 °C Closed**	**45 °C Opened**	**Solution**
0	$11.77 \pm 1.32^{a,A}$	$11.77 \pm 1.32^{a,A}$	$11.77 \pm 1.32^{a,A}$	$11.77 \pm 1.32^{a,A}$	$11.77 \pm 1.32^{a,A}$	$11.54 \pm 0.65^{a,A}$
1	$20.33 \pm 1.17^{c,d,A}$	$19.87 \pm 0.40^{c,d,A}$	$20.36 \pm 0.44^{b,A}$	$20.35 \pm 4.29^{b,c,A}$	$19.16 \pm 0.21^{c,A}$	$20.58 \pm 0.03^{c,A}$
2	$20.06 \pm 0.11^{c,A,B,C}$	$21.07 \pm 0.56^{d,e,C,D}$	$21.51 \pm 0.17^{b,D}$	$19.10 \pm 0.12^{b,c,A}$	$19.49 \pm 1.08^{c,A,B}$	$20.62 \pm 0.13^{c,B,C,D}$
4	$20.24 \pm 0.41^{c,A,B}$	$19.16 \pm 0.08^{c,A}$	$20.62 \pm 1.10^{b,B}$	$19.01 \pm 0.42^{b,c,A}$	$18.87 \pm 0.27^{c,A}$	$23.02 \pm 0.20^{d,C}$
6	$21.94 \pm 0.18^{d,A}$	$22.10 \pm 0.94^{d,A}$	N.D.	$20.86 \pm 0.44^{c,A}$	$21.45 \pm 0.38^{d,A}$	$21.28 \pm 0.27^{c,A}$
10	$16.65 \pm 0.58^{b,A,B}$	$16.90 \pm 0.20^{b,B}$	N.D.	$16.09 \pm 0.09^{b,A}$	$16.96 \pm 0.28^{b,A,B}$	$16.96 \pm 0.18^{b,A,B}$
16	$19.09 \pm 0.15^{c,A}$	$18.95 \pm 0.18^{c,A}$	N.D.	$18.48 \pm 0.33^{b,c,A}$	$19.11 \pm 0.26^{c,A}$	$20.71 \pm 1.07^{c,A,B}$

Lowercase superscripts mean the column followed by different letters are significantly different ($p < 0.05$). Uppercase superscripts mean the row followed by different letters are significantly different ($p < 0.05$). N.D.; not determined.

4. Conclusions

In conclusion, this study assessed the appropriate solvent to extract rambutan rind for use as an antioxidant ingredient in nutraceuticals and cosmetics. Total phenolic and flavonoid content and biological anti-aging activities through antioxidant and tyrosinase inhibition were determined. The extract stability under various conditions in terms of phenolic content and antioxidant activity was also evaluated. The ethanolic extract of rambutan rind contains the highest total phenolic and flavonoid contents, and was the most

effective in the antioxidant activities. In addition, the ethanolic extract of rambutan rind could retain phenolic content higher than 80% and preserve antioxidant activity after the first week along the study period at all storage conditions. Based on the extract's stability, *N. lappaceum* phenolics could be recommended to be used as antioxidant nutraceuticals and anti-aging active ingredients in cosmetics.

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