Antioxidant and antibacterial activities of *Nephelium lappaceum* L. extracts

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Abstract

Ether, methanolic and aqueous extracts of lyophilized rambutan (*Nephelium lappaceum* L.) peels and seeds were evaluated for phenolic contents, antioxidant and antibacterial activities. High amounts of phenolic compounds were found in the peel extracts and the highest content was in the methanolic fraction (542.2 mg/g dry extract). Several potential antioxidant activities, including reducing power, β-carotene bleaching, linoleic peroxidation and free radical scavenging activity, were evaluated. The peel extracts exhibited higher antioxidant activity than the seed extracts in all methods determined (*P* < 0.05). The methanolic fraction was found to be the most active antioxidant as shown by their 50% DPPH/C15 inhibition concentration, 4.94 mg/mL. The results indicated this fraction exhibited greater DPPH radical scavenging activity than BHT and ascorbic acid (0.32 g dry extract/g BHT or ascorbic acid). Antibacterial activity against eight bacterial strains was assessed by disc diffusion and broth macrodilution methods. All peel extracts exhibited antibacterial activity against five pathogenic bacteria. The most sensitive strain, *Staphylococcus epidermidis*, was inhibited by the methanolic extract (MIC 2.0 mg/mL).

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**Keywords**: *Nephelium lappaceum*; Antioxidant; Antibacterial; Phenolics

1. Introduction

Reactive oxygen species (ROS) generated from both living organisms and exogenous sources (Halliwell & Gutteridge, 1999), initiate reactions which damage biological molecules and also play an important causative role in disease initiation (Croft, 1999; Halliwell, 1996). Lipid oxidation, caused by free radicals, is one of the major factors for the deterioration of food products during processing and storage. Effective synthetic antioxidants such as butylated hydroxytoluene (BHT) have been used for industrial processing but these are suspected of being responsible for liver damage and carcinogenesis (Barlow, 1990). Recently, there is an increasing interest in finding natural antioxidants from plant materials to replace synthetic ones.

Plants contain a large variety of substances possessing antioxidant activity, such as vitamin C, vitamin E, carotenoids, xanthophylls, tannins and phenolics (Chanwitheesuk, Teerawutgulrag, & Rakariyatham, 2005). Sources of natural antioxidants are primarily plant phenolics that can be found in all parts of the plant (Pratt, 1992). The plant phenolic compounds, such as flavonoids exhibit antioxidant properties due to their high redox potential (Cook & Samman, 1996). They also exhibit a wide range of biological activity, antimicrobial activity, anticarcinogenicity and antiproliferation, and many biological activities can be attributed to their antioxidant properties (Ren, Qiao, Wang, Zhu, & Zhang, 2003; Tapiero, Tew, Ba, & Mathé, 2002).

Interestingly, recent research has revealed that fruit peels and seeds, such as grape seeds and peels (Jayaprakasha, Selvi, & Sakariah, 2003; Jayaprakasha, Singh, & Sakariah, 2001; Negro, Tommasi, & Miceli, 2003), pomegranate peel (Singh, Murthy, & Jayaprakasha, 2002), sweet orange peel (Anagnopoulou, Kefalas, Papaegiorgiou, Assimopoulou, & Boskou, 2003), and *Nephelium lappaceum* L. peels and seeds, may be promising sources of natural antioxidants.
2006) and mango seed kernel (Kabuki et al., 2000) may potentially possess antioxidant and/or antimicrobial properties. The current study focuses on the possibility of using rambutan peel and seed waste as source of low-cost natural antioxidant and antimicrobial.

2. Materials and methods

2.1. Preparation of extracts

Lyophilized peels and seeds of Nephelium lappaceum L. were powdered and then extracted with ether (three times). The residue was then extracted with methanol (three times) and finally with water (three times). After filtration, the combined ether and methanolic extracts were evaporated under vacuum to absolute dryness and the aqueous extract was lyophilized.

2.2. Determination of the total phenolic contents

The amounts of phenolic compounds in the extracts were determined according to the method of Waterman and Mole (1994) using Folin–Ciocalteu method and catechin was used as the standard phenolic compound. The extract solution in appropriate solvent (0.1 mL) was transferred to a volumetric flask containing 7.9 mL of distilled water. After that, 0.5 mL of Folin–Ciocalteu reagent was added. Three minutes later, 1.5 mL of 200 g/L sodium carbonate solution was added. Subsequently, the shaken mixture was allowed to stand for 2 h at room temperature and then measured at 760 nm. The experiment was carried out in triplicate and the total phenolic compounds were expressed as catechin equivalents.

2.3. Determination of antioxidative activity

2.3.1. Reducing power

The reducing power was based on the method described previously by Yildirim, Mavi, and Kara (2001). Different concentrations of extracts and BHT (Sigma–Aldrich GmbH, Germany) (50–200 μg/mL) in 1 mL of methanol were mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of 10 g/L potassium ferricyanide. The mixture was incubated at 50 °C for 30 min. An aliquot (2.5 mL) of 100 g/L trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of the upper layer solution was mixed with 2.5 mL of distilled water and 0.5 mL of 1 g/L FeCl₃, and the absorbance of the resulting solution was measured at 700 nm.

2.3.2. β-Carotene linoleate model system

The antioxidative activity of rambutan peel and seed extracts was determined according to the method of Jayaprakasha et al. (2001). First, 0.2 mg of β-carotene in 1 mL of chloroform, 20 mg of linoleic acid and 200 mg of Tween-40 were mixed. Chloroform was removed using nitrogen gas and the resulting mixture was topped up to 50 mL using oxygenated water and mixed well. Aliquots (5 mL) of the emulsion were pipetted into different test tubes containing 0.2 mL of extracts in methanol at different concentrations. BHT was used for comparative standard. A control containing 0.2 mL of methanol and 5 mL of the above emulsion was prepared. The tubes were placed at 50 °C in the water bath. Absorbance was taken at zero time (t = 0) at 470 nm. Measurement of absorbance was continued until the color of β-carotene disappeared in the control (t = 180 min) at 15 min intervals. A mixture prepared as above without β-carotene served as blank. The experiment was carried out in triplicate. The antioxidative activity of the extracts was evaluated in terms of the bleaching of β-carotene using the following equation:

\[
\text{Antioxidant activity} = \left[1 - \left(\frac{A_t}{A_0} - \frac{A_t^0}{A_0^0}\right)\right] \times 100
\]

Where \(A_0\) and \(A_0^0\) were the absorbance measured at zero time of the incubation for test sample and control, respectively, and \(A_t\) and \(A_t^0\) were the absorbance measured for the test sample and the control, respectively, after incubation for 180 min. Extract concentration providing 50% inhibition (IC₅₀) was calculated from the graph of inhibition percentage against extract concentration.

2.3.3. Linoleic peroxidation method

The antioxidative activity of rambutan peel and seed extracts was based on the thiocyanate method described previously by Jayaprakasha et al. (2001) with slight modifications. The extracts were dissolved in methanol for stock solutions. BHT was used as comparative standard. The solution containing stock extract solution or BHT at various concentrations in 2.5 mL of potassium phosphate buffer (0.04 mol/L, pH 7.0) was transferred to 2.5 mL of linoleic acid emulsion containing 0.28 g Tween-40, 0.28 g linoleic acid dissolved in potassium phosphate buffer (0.04 mol/L, pH 7.0). A control, containing 2.5 mL linoleic acid emulsion and 2.5 mL potassium phosphate buffer (0.04 mol/L, pH 7.0) was prepared. The mixture was incubated at 37 °C for 64 h. Aliquots (0.1 mL) were drawn from the incubation mixture at 8-h intervals and then mixed with 5.0 mL of 75% (v/v) ethanol, 0.1 mL of 300 g/L ammonium thiocyanate and 0.1 mL of 20 mmol/L ferrous chloride in 0.96 mol/L HCl and allowed to stand at room temperature for 3 min. The absorbance was measured at 500 nm. The experiments on antioxidative activity were run in triplicate and the percent inhibition of lipid peroxidation was calculated using the following formula:

\[
\text{Percent inhibition} = 100 - \left[\frac{A_t}{A_0} \times 100\right]
\]

Where \(A_0\) was the absorbance of the control reaction and \(A_t\) was the absorbance in the presence of the extracts. The antioxidative activities were illustrated as IC₅₀ values.

2.3.4. DPPH radical scavenging activity

The stable free radical scavenging activity was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Sigma–Aldrich GmbH, Germany) method of Gülçin, Oktay, Kireçci, and Kürşü (2003). A 0.1 mmol/L DPPH solution in methanol was prepared, and then 1 mL of this solution was
mixed with 3 mL of extract at different concentrations (0.78—400 µg/mL). A control, containing 1 mL of DPPH solution and 3 mL of methanol was prepared. The mixture was incubated at room temperature for 30 min and then the absorbance was measured at 517 nm. The ability to scavenge the DPPH radical was calculated as percent DPPH scavenging using the following equation:

$$\text{%DPPH} \text{ scavenging} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100$$

Where $A_0$ was the absorbance of the control and $A_1$ was the absorbance of the mixture containing extracts. IC$_{50}$ of reference antioxidant compounds, BHT, quercetin and ascorbic acid (Sigma—Aldrich GmbH, Germany), were used for comparison to IC$_{50}$ of the extracts.

### 2.4. Bacterial cultures

The microorganisms used in this study consisted of eight strains of pathogenic bacteria, viz. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholerae*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. All bacterial strains were obtained from the Department of Clinical Microbiology, Faculty of Associated Medical Sciences, Chiang Mai University. The bacteria were grown and maintained on nutrient agar slants. The inoculated agar slants were incubated at 37 °C.

### 2.5. Antibacterial assays

#### 2.5.1. Disc diffusion assay

The antibacterial activity was based on disc diffusion method (Bauer, Kirby, Sherris, & Truck, 1966) using bacterial cell suspension whose concentration was equilibrated to a 0.5 McFarland standard. A 100 µL of each bacterial suspension was spread on a Mueller—Hinton agar plate. Sterile paper discs (6 mm diameter) were impregnated with 20 µL of each extract dissolved in the solvent used for extraction at 125 mg/mL. The discs were allowed to dry and then placed on the inoculated agar. Discs with the solvent used for dissolution were used as negative control and 10 µg streptomycin discs were used as positive controls. The plates were incubated at 37 °C for 24 h. After incubation time, zone of inhibition was measured. The experiment was performed in triplicate.

#### 2.5.2. Minimum inhibition concentrations (MICs)

Minimum inhibition concentrations of the extracts were evaluated for the bacterial strains which were determined as being sensitive to the extracts in the disc diffusion assay. A broth macrodilution method was used, as previously described by Nakamura et al. (1999) with a slight modification. Serial twofold dilutions of each extract were prepared in 10% (v/v) dimethylsulfoxide (DMSO), and 30 µL of each dilution was added to 3 mL of Mueller—Hinton broth. These were inoculated with 30 µL of culture of the test bacterial strains. After incubation of the cultures at 37 °C, the MIC value was determined as the lowest concentration of the extract that demonstrated no visible growth.

### 2.6. Statistical analysis

All experimental results were expressed as means ± S.D. Analysis of variance was performed by ANOVA procedures. Correlation coefficient ($R$) was used to determine two variables. SPSS software was used for statistical calculations. The results with $P < 0.05$ were regarded to be statistically significant.

### 3. Results and discussion

In this study, phenolic content, antioxidant activity and antibacterial activity of various *N. lappaceum* peel and seed extracts were determined. The extraction yields (g/100 g lyophilized rambutan) from various extractants, i.e. ether, methanol and water are presented in Table 1. The total phenolic contents of the extracts were determined by Folin—Ciocalteu method. The high amounts of phenolic compounds were found in the peel extracts in the following order: methanolic fraction (542.2 mg/g), aqueous fraction (393.2 mg/g) and ether fraction (293.3 mg/g) (Table 1). Although low amounts of phenolic compounds were observed in the seed extracts, the methanolic fraction of seed extract contained the highest phenolic content (58.5 mg/g).

The extracts obtained by various solvent extractions were determined for their antioxidant activities. The extracts were investigated for the reductive capabilities by using the potassium ferricyanide reduction method. The reducing ability may serve as a significant indicator of potential antioxidant activity (Meir, Kanner, Akiri, & Hadas, 1995). The peel and seed extracts increased in reducing powers with increasing concentration (Fig. 1) and the peel extracts exhibited higher reducing ability than the seed extracts. The methanolic extract showed the highest activity, followed by the aqueous and ether extracts. When compared to the BHT, the methanolic fraction showed significantly ($P < 0.05$) higher activity at all concentrations. Yen and Chen (1995) reported that the extract which

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Extraction yields and total phenolic content of <em>Nephelium lappaceum</em> L. extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extraction yield (g/100 g)</td>
</tr>
<tr>
<td><strong>Peel extracts</strong></td>
<td></td>
</tr>
<tr>
<td>Ether</td>
<td>2.84</td>
</tr>
<tr>
<td>Methanol</td>
<td>25.1</td>
</tr>
<tr>
<td>Aqueous</td>
<td>2.06</td>
</tr>
<tr>
<td><strong>Seed extracts</strong></td>
<td></td>
</tr>
<tr>
<td>Ether</td>
<td>21.2</td>
</tr>
<tr>
<td>Methanol</td>
<td>4.80</td>
</tr>
<tr>
<td>Aqueous</td>
<td>7.29</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D.

$^a$-$^e$Means the column followed by different letters are significantly different ($P < 0.05$).

$^f$ Express as mg catechin/1 g dry extract.
showed a reducing power could function as an electron donor and also could reduce the oxidized intermediates generated from the lipid peroxidation reaction.

For their antioxidant activity, the extracts were determined for their capability to delay β-carotene bleaching in the coupled oxidation of β-carotene/linoleic acid model system. Fig. 2 presents the antioxidant activity of the extracts and BHT. Like the reducing power, high activity was found in the peel extracts and lower activity was found in the seed extracts. The methanolic and aqueous fractions of the peel showed potent antioxidative activity with no statistical difference \((P > 0.05)\) when compared to the antioxidant standard, BHT. The total antioxidant activity by lipid peroxidation based on ferric thiocyanate method was also evaluated. The peel extracts still showed statistically higher activity than that of the seed extracts \((P < 0.05)\). The antioxidant activity (IC\(_{50}\)) of the peel extracts is ranked as follows: methanolic fraction \((0.46 \mu g/mL)\) > aqueous fraction \((0.55 \mu g/mL)\) > ether fraction \((0.81 \mu g/mL)\) (Fig. 3).

The free radical scavenging capacity of the extracts against common free radicals (DPPH') \textit{in vitro} were further determined. Fig. 4 shows the dose-response curve of DPPH' scavenging activities of the extracts. The results indicated that the peel extracts exhibited a potential free radical scavenging activity. The half inhibition concentration (IC\(_{50}\)) of the radical scavenging activity of the peel extracts were calculated and are illustrated in Table 2. The results revealed that the extract with the highest effective radical scavenging activity was the methanolic fraction \((4.94 \mu g/mL)\), followed by the aqueous extract \((9.67 \mu g/mL)\) and the ether extract \((17.3 \mu g/mL)\), while lower activities were found in the seed extracts \((>400 \mu g/mL)\) (data not shown). BHT and ascorbic acid revealed less effective activities \((P < 0.05)\) than the methanolic extract \((0.32 \text{ mg extract/mg reference compounds})\) and the aqueous extract \((0.64 \text{ mg extract/mg reference compounds})\), while quercetin showed stronger radical scavenging activity than these two extracts \((>1 \text{ mg extract/mg quercetin})\). The ether fraction
showed the lowest activity among peel extracts and antioxidant standards. According to these results, the antioxidant activity increased proportionally with the phenolic content. Correlations between phenolic content and antioxidant activity were investigated. There was a substantial correlation between the phenolic content versus reducing power ($R^2 = 0.96$), antioxidant activity as inhibition of β-carotene bleaching ($R^2 = 0.61$), antioxidant activity as thiocyanate method ($R^2 = 0.68$) and free radical scavenging activity ($R^2 = 0.96$). Thus, it can be noted that the strong antioxidant properties may be attributed to the phenolic components in the extracts. The significant correlation between the phenolic content and the antioxidant activity of various vegetable extracts has been previously observed (Velioglu, Mazza, Gao, & Oomah, 1998). There are many types of compounds possessing antioxidant activity in higher plants (Larson, 1988) and the phenolic compounds were highlighted to be the potential antioxidants (Yu et al., 2005). The fact that phenolic compounds possess a high potential to scavenge radicals can be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl groups (Sawa, Nakao, Akaide, Ono, & Maeda, 1999). The inhibitory effects on lipid peroxidation and autoxidation of linoleic acid have been attributed to the radical scavenging activity (Hatano et al., 2005).

The antibacterial activity of the peel and seed extracts of *N. lappaceum* L. were determined against eight strains of pathogenic bacteria as shown in Table 3. The solvents used for control and all rambutan seed extracts did not show any activity (the results not shown). As shown in Table 3, all rambutan peel extracts exhibited a potent activity against the tested bacteria except *E. coli*, *K. pneumoniae* and *S. typhi*, and only the ether extract did not inhibit the growth of *P. aeruginosa*. To compare the sensitivity of the bacterial strains to the extracts, the extracts that exhibited antibacterial activity in disc diffusion assay were submitted to the minimum inhibition concentration (MIC) test. The MIC values are illustrated in Table 4. There were substantial differences between the MICs of various extracts. The methanolic fraction had the most effective antibacterial activity. The bacterium *S. epidermidis* was the most sensitive strain to the methanolic extracts of *N. lappaceum* peel (MIC 2.0 mg/mL). These results verify the earlier studies that methanol is the better solvent for more consistent extraction of antimicrobial substances compared to the other solvents (Ahmad, Mehmood, & Mohammad, 1998; Natarajan et al., 2005).

Similar to antioxidant activity, only fractions containing a high phenolic content of the peel extracts exhibited the antibacterial activity. Phenolic compounds have also been reported

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**Table 2**
The 50% inhibition concentrations (IC$_{50}$) for DPPH radical scavenging activity of *Nepelhium lappaceum* L. peel extracts and comparison with the four reference antioxidant compounds: BHT, quercetin, α-tocopherol, and ascorbic acid.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>IC$_{50}$ (µg/mL)</th>
<th>Dry extract/mg reference compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether</td>
<td>17.3 ± 1.03</td>
<td>1.14 ± 0.10</td>
</tr>
<tr>
<td>Methanol</td>
<td>4.94 ± 0.26</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>Aqueous</td>
<td>9.67 ± 0.87</td>
<td>0.64 ± 0.07</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D.

BHT; butylated hydroxytoluene.

**Table 3**
Antimicrobial activity of the extracts of *Nepelhium lappaceum* L. peel (2.5 mg/disc) against the tested microorganisms based on disc diffusion method.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Inhibition zone in diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ether</td>
</tr>
<tr>
<td><strong>Gram negative bacteria</strong></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>–</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>–</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>–</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>–</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>12.25 ± 1.31</td>
</tr>
<tr>
<td><strong>Gram positive bacteria</strong></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>7.50 ± 0.41</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>7.00 ± 0.29</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>10.00 ± 0.20</td>
</tr>
</tbody>
</table>

Values are means ± S.D (mm) of three separate experiments; “–”, no inhibition zone.

**Table 4**
The Minimum inhibition concentration (MIC) values of the extracts of *Nepelhium lappaceum* L. peel extract.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Ether extract (mg/mL)</th>
<th>Methanol extract (mg/mL)</th>
<th>Aqueous extract (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram negative bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>ND</td>
<td>62.5</td>
<td>125</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>62.5</td>
<td>15.6</td>
<td>15.6</td>
</tr>
<tr>
<td><strong>Gram positive bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>62.5</td>
<td>15.6</td>
<td>62.5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>31.2</td>
<td>31.2</td>
<td>31.2</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>31.2</td>
<td>2.0</td>
<td>15.6</td>
</tr>
</tbody>
</table>

ND: not determined.
to be responsible for antimicrobial properties. Penna et al. (2001) isolated two antibacterial phenolic compounds, methyl gallate (MIC 128 µg/mL) and protocatechuic acid (MIC 128 µg/mL) from *Sebastiania brasiliensis*. Seven antibacterial phenolic compounds, viz. ethyl gallate, caffeic acid, dihydrokaempferol, eriodictyol, quercetin, 3′,5′,7-pentahydroflavanone and (−)-epicatechin were isolated from *Gleditsia sinensis* Lam. (Zhou, Li, Wang, Liu, & Wu, 2007). Thus, it may be concluded that the phenolic compounds in the *N. lappaceum* extracts could be the main components which possess the antioxidant and antibacterial properties.

4. Conclusion

This study has demonstrated the antioxidant and antibacterial activities of various extracts from *N. lappaceum* L. peel and seed parts. More potential activities were found in the peel extracts than the seed extracts. Methanol was a better solvent for extraction of antioxidant and antibacterial substances compared to the other solvents by providing high extraction yields and also strong antioxidant and antibacterial activities. The activities determined in the extracts could be attributed to the phenolic components. Thus, the *N. lappaceum* L. peel can be considered as an easily accessible source of natural antioxidants and antibacterial agents. We are continuing our efforts to identify the antioxidative and antibacterial phenolic compounds in the methanolic fraction of *N. lappaceum* L. peel by further fractionation and analysis.

Acknowledgement

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