Identification of *Nephelium lappaceum* leaves phenolic and flavonoid component with radical scavenging, antidiabetic and antibacterial potential

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*Nephelium lappaceum* Linn. (Rambutan) is traditionally claimed, as a source of natural antioxidants and for its use in the treatment of diabetes and bacterial infections. The present study investigates the *in vitro* effect of ethanolic Rambutan leaves extract (NL) for its antioxidant effect, α-glucosidase, α-amylase enzyme inhibition, and antibacterial potentials. The total phenolic, total flavonoid content of NL was quantified and were expressed in terms of gallic acid (19.6±0.04 mg GAE/g) and rutin equivalents (16.7±0.01 mg RUE/g) respectively. The antioxidant assay revealed that NL exhibited significant inhibition of DPPH (IC 50±SEM: 1.52±0.03 μg/mL) and ABTS (IC 50±SEM: 1.295±0.05 μg/mL) radicals. NL also inhibited both α-amylase (IC 50±SEM: 2.624±0.07 μg/mL), α-glucosidase (IC 50±SEM: 2.416±0.06 μg/mL) enzyme activities, supported by its antioxidant potential and its phenolic and flavonoid content. The antibacterial activity was screened against seven human pathogenic ATCC strains for which the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were recorded. The selected MIC dose was tested, confirmed by Kirby-Bauer agar well diffusion method. NL exhibited MIC and MBC of 62.5 μg/mL and 125 μg/mL against *B. subtilis* and *E. coli* respectively. The results support the scientific claim of NL for its antioxidant, antidiabetic and antibacterial potential.

**Keywords:** Antibacterial, Free Radical Scavenging, *Nephelium lappaceum*, Rambutan, α-amylase, α-glucosidase.

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The plant Rambutan (*Nephelium lappaceum*. Linn.) has been traditionally used in many Asian countries for several therapeutic purposes. The vast majority of the developing nations have adopted a traditional therapeutic practice as a vital piece of their way of life. Historically, most therapeutic preparations were obtained from plants, and used either as a crude extract or as in the refined form. The journey for the new compound isolation from traditional plants has become an alluring research area. Plants with ethnopharmacological significance were being considered on account of their medicinal values and additionally for their proficient medicinal properties.

Rambutan falls under the Sapindaceae family and it is cultivated in the tropical regions such as Malaysia, Indonesia and Thailand. Rambutan has many traditional uses and all its parts including its leaves, rind and seeds were reported to contain important phytochemical constituents. Phenolic compounds were the most important phytochemical constituent investigated in Rambutan fruit peels and were reported to possess antioxidant, antibacterial, antidiabetic and α-amylase inhibitory properties. The Rambutan leaves are not fully explored, for their medicinal properties and hence the need arises as this tropical plant is abundant in many Asian countries, its medicinal properties can be explored further. In this context, we planned to analyse the antioxidant,
antidiabetic and antibacterial potential of ethanolic Rambutan leaves extract (NL).

**Methodology**

**Chemicals**

DPPH, α-amylase, α-glucosidase, Ascorbic Acid, Gentamycin and Gallic acid were purchased from Merck Millipore Corporation, USA. ABTS, Acarbose and Rutin were purchased from Sigma-Aldrich Corporation, USA. All the chemicals used were of analytical grade.

**Collection of sample**

The leaves of *N. lappaceum* were collected from Baling, 09100, Kedah, Malaysia. The matured and healthy plant was selectively chosen for sampling. The leaves were brought into the laboratory in sterile bags and processed within a few hours after sampling. The plant specimen was authenticated at the Department of Pharmacognosy, AIMST University, Malaysia (Ref. No.: AIMST/FOP/04).

**Extract preparation**

The leaves were cleaned under running tap water and air-dried for 2 days to avoid any chemical decomposition from taking place. 200 g of dried leaves were then finely grounded and macerated using 500 mL of 70% ethanol in a 1 L flask. The sample was macerated and kept on the shaker for 5 days at room temperature. The sample was filtered through a muslin cloth and the process was repeated until a clear colorless supernatant liquid obtained. Filtrates were collected and evaporated using a rotary evaporator.

**Qualitative phytochemical screening**

The ethanolic Rambutan leaves extract (NL) was used for preliminary screening of phytochemicals such as alkaloids, carbohydrates, phenols, amino acids, steroids, anthocyanins, proteins, flavonoids, saponins, mucilage, gums, glycosides and tannins using standard biochemical testing methods.

**Quantification of total phenolic content**

Total phenolic content of the NL was analyzed according to the protocol described. Various concentrations of NL were prepared as mentioned in the total phenolic content. A reaction mixture containing 1 mL of NL, 10 mL of 30% ethanol and 0.7 mL of 5% sodium nitrite and 10% aluminium chloride was prepared and incubated at room temperature for 6 min. Followed by the addition of 10 mL of 1 M sodium hydroxide solution, the final volume was made up to 25 mL with 30% ethanol and kept for 10 min. The absorbance was measured at 450 nm. The procedure was repeated for rutin (RU) as a standard and the calibration curve was constructed. The flavonoid concentration was expressed as rutin equivalents (RUE, mg/g).

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**Antioxidant assay**

**DPPH radical assay**

The ability of NL to scavenge DPPH radical was assessed using the procedures described earlier with minor modifications. Different concentrations of NL were prepared using 70 % ethanol. A reaction mixture containing 0.5 mL of NL (10 to 1000 µg/mL), 0.5 mL of 2 µM DPPH were mixed and incubated for 20 min at room temperature. The absorbance was measured at 517 nm and the above procedure was repeated for the standard ascorbic acid. The percentage of scavenging was calculated using the equation (Eq 1).

\[
\text{% Inhibition} = \left(\frac{\text{Absorbance Control} - \text{Absorbance Sample}}{\text{Absorbance Control}}\right) \times 100
\]  

(Eq. 1)

**ABTS radical cation decolourization assay**

The 2, 2’-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS⁺⁺) free radical cation scavenging ability of NL was assessed following the procedures described earlier. Various concentrations of NL were prepared as mentioned in DPPH assay. A reaction mixture containing 0.5 mL of NL, 0.5 mL of ABTS (7 mM) were mixed and incubated for 30 min at room temperature. The absorbance was measured at 750 nm. The procedure was repeated for gallic acid (GA) as a standard and the calibration curve was constructed. Based on the absorbance values, the phenolic concentration was expressed as mg/mL from the calibration curve. The total phenolic content of NL was expressed as gallic acid equivalents (GAE, mg/g).
at room temperature and the absorbance was measured at 734 nm. The procedure was repeated for the standard ascorbic acid and the percentage of scavenging calculated using the equation (Eq. 1).

Antibacterial assay

Microbial inoculum standardization

The following bacterial strains used were *Bacillus subtilis* (ATCC 11774), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), *Streptococcus pyogenes* (ATCC 19615), *Neisseria gonorrhoeae* (ATCC 43069), *Pseudomonas aeruginosa* (ATCC 10145), and *Escherichia coli* (ATCC 10799). The strains were placed on Mueller Hinton agar plates at 36°C and adjusted to 0.5 McFarland standard.14

Determination of minimum inhibitory and bactericidal concentration

The minimum inhibitory concentration (MIC) was determined using the test tube dilution method. Nutrient broth (1 mL) was added into sterile tubes. Subsequently, 1 mL of 1 mg/mL extract was transferred in a tube and serially diluted to produce concentrations ranging from 31.25 µg/mL to 500 µg/mL. 0.1 mL of inoculum was inoculated into each test tube and incubated for 24 h at 37°C and the growth of microorganisms was monitored by the turbidity produced. 10% DMSO was used as solvent. MIC was determined with the lowest concentration of a sample that suppresses the bacterial growth.15

The minimum bactericidal concentration (MBC) was determined by comparing the number of viable bacteria or inoculum with the initial number. Clear tubes from MIC study were diluted further and spread onto a nutrient agar plate and incubated for 24 h at 37°C.

Kirby-Bauer Agar well diffusion method

The selected bacterial inoculum was spread evenly onto a fresh Mueller Hinton agar plates with the help of a sterile cotton swab. 4 wells sized 12 mm in diameter were made into the agar plate and 50 µL of the extract was added to each well. These plates were incubated for 24 h at 37°C, under aerobic condition. After the incubation period, the diameter of inhibition zones was measured. Gentamycin and 10% DMSO were used as positive and negative controls, respectively.15

In vitro antidiabetic assay

α-Amylase inhibitory assay

A reaction mixture containing 0.5 mL (100-1000 µg/mL) of NL and 0.5 mL of α-amylase solution (0.5 mg/mL) in 0.2 mM phosphate buffer (pH 6.9) was incubated for 10 min at 25°C. 0.5 mL starch solution (1% w/v) in 0.02 M sodium phosphate buffer (pH 6.9) was added. The samples were incubated at 25°C for 10 min and 1 mL of dinitrosalicylic acid (color reagent) was added. The reaction mixture was boiled for 5 min, cooled to room temperature followed by addition of 10 mL of distilled water. The absorbance was measured at 540 nm. Acarbose was used as the standard drug and the percentage of inhibition was calculated using the equation (Eq. 1).

α-Glucosidase inhibitory assay

A volume of 1 mL α-glucosidase (1 U/mL) was pre-incubated with 1 mL of NL (100-1000 µg/mL) for 5 min at 37°C. Then, 1 mL of 2% sucrose in Tris buffer (pH 8) was added and incubated for further 20 min. The reaction was arrested by heating in boiling water for 2 min. Subsequently, the liberated glucose was measured by the GOD-POD method, where acarbose was used as the standard drug.17

Statistical analysis

The inhibitory concentration 50% (IC50) values were computed using the Graph Pad Prism Software (Version 5) by non-linear regression graph plotted between percentage of radical scavenging (x-axis) against concentration (y-axis) for antioxidant studies and percentage of enzyme inhibition (x-axis) versus concentration (y-axis) for antidiabetic studies.

Results and Discussion

Qualitative phytochemical screening

The phytochemical results showed evidences for the presence of various phytoconstituents such as reducing sugar, monosaccharides, carbohydrates, phenols, proteins, tannins, alkaloids, flavonoids, steroids, saponins and glycosides. The flavonoid content present in NL was determined from the standard Rutin curve as portrayed in Fig. 1 (a). The linear regression equation of y = 0.0003x + 0.025 and a high degree of correlation (R² = 0.987) amongst concentration and the absorbance were depicted from the calibration curve as shown in Fig. 1 (a). Thus, the total flavonoid content of NL was found to be 16.7 ± 0.01 mg RUE/g of crude extract.

The phenolic content of the NL was determined from the standard GA curve as portrayed in Fig. 1 (b). A high correlation (R² = 0.9665) amongst concentration and as the absorbance was depicted from the standard calibration curve, which showed the
linear regression equation of $y = 0.0009x + 0.0779$. Thus, total phenolic content of NL is found to be $19.6 \pm 0.04$ mg GAE/g of crude extract.

The aluminum chloride colorimetric technique was utilized to quantify the flavonoid content. The phenolic content of NL was measured by Folin–Ciocalteu technique. Under alkaline conditions, phenols able to dissociate the reagent into a proton and phenolate anion or by electron transfer. The phenolate anion decreases the Mo (VI) of Folin's reagent prompting the development of blue coloured complex with absorption taken at 750 nm. The outcomes uncover that the phenols in NL are profoundly polar in nature and subsequently ethanol could be the best dissolvable solvent to recover it. The earlier research findings also supports that polyphenols and flavonoids of Rambutan extract contributes for their antioxidant properties\textsuperscript{6-7}.

**Antioxidant assay**

Fig. 2 (a) and 2 (b) showed the DPPH and ABTS radical scavenging ability of NL and ascorbic acid. At the highest concentration (1000 μg/mL), NL exhibited 89.6% and 85.81% of inhibition of DPPH and ABTS free radicals respectively. The IC$_{50}$ values of NL was found to be 1.52 ± 0.03 and 1.295 ± 0.05 μg/mL for DPPH and ABTS assays respectively. Similarly, the standard ascorbic acid showed an IC$_{50}$ value of 0.93 ± 0.1 and 1.074 ± 0.09 μg/mL.

From the earlier research findings, it is clear that the hydroxyl moiety of Rambutan phenolic compound possesses radical scavenging properties and reduces lipid peroxidation capacity of cells\textsuperscript{18}. Numerous polyphenolic constituents obtained from plants are more viable antioxidants and accordingly may contribute to protective impacts \textit{in vivo}.

**Antidiabetic assay**

Table 1 showed α-amylase and α-glucosidase inhibitory effect of NL and standard acarbose. At the highest concentration (1000 μg/mL), NL exhibited 80 % and 86.8 % of inhibition of α-amylase and α-glucosidase respectively. The IC$_{50}$ values of NL was found to be 2.62 ± 0.07 and 2.42 ± 0.06 μg/mL for α-amylase and α-glucosidase respectively. Similarly, the standard acarbose exhibited IC$_{50}$ values 2.25 ± 0.15 2.22 ± 0.04 μg/mL.

The pancreatic α-amylase is one of the vital enzymes in the human digestive system, as it
breakdowns starch into oligosaccharides and disaccharides. The liberated glucose, which is later absorbed into the blood circulation. The inhibition of carbohydrate hydrolyzing enzymes would lessen the starch breakdown in the intestinal tract. Subsequently, the postprandial hyperglycaemia level may reduce19. According to previous studies 20, the phytoconstituents with strong antioxidant ability have been known to be good enzyme inhibitors, hyperglycemia regulators and ameliorate diabetic complications resulting from oxidative stress. The results showed that the NL had comparable potential inhibitors of α-amylase and α-glucosidase when compared with acarbose, this could be attributed due to its flavonoid, phenolic contents and antioxidant property.

Antibacterial assay

The MIC and MBC results of NL were depicted in Table 2. NL exhibited the highest MIC at 62.5 µg/mL and MBC at 125 µg/mL for both B.subtilis and E.coli. At MIC of 125 µg/mL, as shown in Table 3, NL portrayed significant zone of inhibition against B.subtilis, 13.7±0.58 mm and E.coli, 13.3±0.58 mm compared to gentamycin 14.0 ± 1.01 mm, 13.7±0.58 mm respectively (Fig. 3). NL showed moderate antibacterial activity (E.faecalis and S.aureus) and least antibacterial activity (S.pyogenes and N.gonorrhoeae) among the tested ATCC bacterial strains. Rambutan peel extracts were reported earlier for its antibacterial8 potential and it’s the first report to reveal the antibacterial effect of Rambutan leaves. Phytochemicals for instance sesquiterpenes, terpenoids, tannins, phenolic, flavonoids, polyphenols are known to act against a broad spectrum of microbes11. The study revealed that NL is a conceivable antibacterial agent comparable to standard gentamycin. The antibacterial activity of NL could be credited due to its high phenolic and flavonoid content. These phytochemical constituents have been accounted to hinder the nucleic acid synthesis and other metabolic activities21. The phenolic constituents with C3 side chain at lower oxidation level and the oxygen absences have also reported to be responsible for the antibacterial effect. The toxicity of polyphenol’s mechanism against virulent microbes could be related to the hydrolytic enzyme inhibition particularly proteases or diverse

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC (µg/mL)</th>
<th>MBC (µg/mL)</th>
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<tbody>
<tr>
<td>B.subtilis</td>
<td>62.5</td>
<td>125.0</td>
</tr>
<tr>
<td>E.faecalis</td>
<td>125.0</td>
<td>500.0</td>
</tr>
<tr>
<td>S.aureus</td>
<td>125.0</td>
<td>500.0</td>
</tr>
<tr>
<td>S.pyogenes</td>
<td>125.0</td>
<td>500.0</td>
</tr>
<tr>
<td>N.gonorrhoeae</td>
<td>250.0</td>
<td>1000.0</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>250.0</td>
<td>1000.0</td>
</tr>
<tr>
<td>E.coli</td>
<td>62.5</td>
<td>125.0</td>
</tr>
</tbody>
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Values are expressed as Mean ± Standard deviation (SD), (n=3).
interactions that inactivate the microbial adhesion, cell envelope transport proteins and non-specific interactions with sugars\textsuperscript{22}.

Conclusion
The outcomes of the study uncover the significant free radical scavenging ability, antidiabetic and antibacterial potential present in the leaves of *Nephelium lappaceum*. Henceforth, these findings show that NL could be a rich natural source for bioactive metabolites as potential pharmacological agents. However, further studies are required to elucidate and comprehend these potentials using different biochemical and molecular biology tools to explore the complete mechanisms.

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