

Inhibitory Potential of the Rambutan Rind Extract and Tannin against Alpha-Amylase and Alpha-Glucosidase Activities *in vitro*

Aree Thinkratok, Nattapon Supkamonseni, and Rungrudee Srisawat*

Abstract—This study investigated the effects of the rambutan rind extract and tannin (one of phenolic compounds found in the rambutan rind extract) on alpha-amylase and alpha-glucosidase activities *in vitro*. The rambutan rind extract and tannin (at the doses up to 20 mg/ml) showed potent inhibitory effects on both alpha-amylase and alpha-glucosidase activities *in vitro*. The maximum percentage inhibition on alpha-amylase enzyme activity by the rambutan rind extract and tannin were obtained at concentration of 2.50 mg/ml (97.30% and 95.65%, respectively), while the maximum percentage inhibition of alpha-glucosidase enzyme activity on the rambutan rind extract and tannin were obtained at 2.50 mg/ml (96.66%) and 5 mg/ml (95.79%), respectively. The present findings suggested that the rambutan rind extract and tannin displayed beneficial effects in the treatment of type 2 diabetes mellitus, possibly by alpha-amylase and alpha-glucosidase activities inhibition.

Keywords—alpha-amylase, alpha-glucosidase, rambutan rind extract, tannin

I. INTRODUCTION

DIABETES mellitus is a chronic metabolic disorder that is characterized by high blood glucose level (hyperglycemia) resulting from defects in insulin production, insulin action, or both. Management for diabetes mellitus, in particular type 2 diabetes mellitus (non-insulin-dependent diabetes mellitus), can be achieved by decreasing postprandial hyperglycemia. The important strategy for managing postprandial hyperglycemia is to prevent glucose absorption by the inhibition of carbohydrate-hydrolyzing enzymes, such as alpha-amylase and alpha-glucosidase, resulting in decrease the postprandial elevation of blood glucose after a mixed carbohydrates diet [1]–[4]. Many available synthetic drugs such as acarbose, voglibose, and miglitol are widely used as

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inhibitors of these enzymes in patients with type 2 diabetes [5]–[6]. These chemical drugs have strong inhibitory effects on both alpha-amylase and alpha-glucosidase activities, however, their several side effects have been reported, such as liver disorders, flatulence, abdominal pain, renal tumors, and diarrhea [7]–[8]. It becomes necessary to identify the alpha-amylase and alpha-glucosidase inhibitors from natural sources that have lesser side-effects.

Rambutan (*Nephelium lappaceum* L.) fruit rind is traditionally used for treatment of diarrhea, dysentery, and fever [9]. Rambutan rind extract was found to contain ascorbic acid and high levels of phenolic compounds (anthocyanins, flavonoids, tannins, ellagic acid, corilagin, and geraniin) [10]–[13] and possess antioxidant, antibacterial, anti-herpes simplex virus type 1, antiproliferative, and anti-hyperglycemic activities [10]–[11], [14]–[17]. Therefore, the present study was designed to evaluate the inhibitory potential of the crude extract from the fruit rind of rambutan and tannin (one of phenolic compounds found in the rambutan rind extract) against alpha-amylase and alpha-glucosidase activities *in vitro*.

II. MATERIALS AND METHODS

A. Preparation of Plant Extract

Rambutan (*Nephelium lappaceum* L.) fruits were obtained from local market in Nakhon Ratchasima province during June-August 2008. The plants were authenticated as *Nephelium lappaceum* L. at the Forest Herbarium, Department of National Parks, Wildlife and Plant conservation and a voucher specimen (BKF 185498) was deposited in the Herbarium.

The rind of rambutan was washed with copious amounts of water and dried at room temperature for 2-4 days. The dried rind was powdered using an electric mill. The powder was macerated with 85% hydroethanolic solution at room temperature for 7 days. The obtained suspension was filtered and concentrated using a rotary evaporator and then converted into crude extract by freeze dryer. The obtained crude extract was stored at -20 °C until further used.

B. Phenolic Content Analysis and Antioxidant Activity of the Rambutan Rind Extract

- Determination of Total Phenolic Content of the Rambutan Rind Extract

The total phenolic compounds of the rambutan rind extract were measured according to the Folin-Ciocalteu reagent method that was adapted from the method of Minussi et al. [18]. Briefly, the rambutan rind extract was dissolved in 10% ethanol. The reaction mixtures consisted of 200 μ l of the rambutan rind extract solution and 4 ml of 2% sodium carbonate (Na_2CO_3) were mixed. Two minutes later, 200 μ l of Folin-Ciocalteu reagent was added and incubated at room temperature for 30 min. The mixtures were then measured absorbance by using a spectrophotometer at 750 nm. The total phenolic compounds were expressed as gallic acid equivalents (GAE) in milligrams per gram of dry extract. All determinations were performed in triplicate.

- Determination of Antioxidant Activity of the Rambutan Rind Extract

The free radical scavenging activity of the rambutan rind extract was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method that was adapted from the method of Blois [19]. Briefly, 4 ml of 0.5 mM DPPH in methanol, 800 μ l of the rambutan rind extract at different concentrations and 4 ml of 0.1 M tris HCl buffer (pH 7.0) were added in the test tubes. After that, the mixtures were then mixed and incubated at room temperature for 30 min. The mixtures were measured absorbance by using a spectrophotometer at 517 nm. All determinations were performed in triplicate. The ability to scavenge the DPPH radical was calculated as percent DPPH scavenging using the following equation:

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

Where: A_0 is the absorbance of the control and A_1 is the absorbance of the mixture containing extracts. The DPPH radical scavenging activity (%) was plotted against the plant extract concentration (mg/ml) to determine the concentration of extract necessary to decrease DPPH radical scavenging by 50% inhibition concentration (IC_{50}).

C. Methods for Studying Enzyme Inhibition in vitro

- In vitro Assay for Measuring the Inhibition of Alpha-Amylase Enzyme

Alpha-amylase activity was determined by colorimetric method using starch as the substrate. This method was adapted from method of Nickavar, Abolhasani, and Izadpanah [20]. Briefly, the rambutan rind extract, tannin, and a positive control acarbose were dissolved in DDD water to give concentrations ranging from 0.15626 to 20 mg/ml, 1.25 to 20 mg/ml, and 10 mg/ml, respectively. The reaction mixtures consisted of 50 μ l the rambutan rind extract, tannin,

or acarbose and 50 μ l of 0.01% (w/v) alpha-amylase enzyme solution were mixed in microtube and incubated in water bath at 25 °C for 30 min. After that, 100 μ l of 1% (w/v) starch solution was added to these mixtures, mixed and incubated in water bath at 25 °C for 3 min. The color reagent solution (100 μ l) was added to these mixtures. The mixtures were then mixed and incubated in water bath at 85 °C for 15 min. After that, the mixtures were removed from the water bath and allowed to ice-cooled. Thereafter, 900 μ l of DDD water was added to the mixtures. The absorbance of the assay mixture was measured by Benchmark Plus Microplate Spectrophotometer at 540 nm. All determinations were performed in triplicate. The inhibition percentage of alpha-amylase enzyme was assessed by following formula:

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

$$\Delta A_{\text{Control}} = A_{\text{Test1}} - A_{\text{Blank1}}$$

$$\Delta A_{\text{Sample}} = A_{\text{Test2}} - A_{\text{Blank2}}$$

Where: A_{Test1} and A_{Test2} were defined as the absorbance of DDD water and sample with alpha-amylase enzyme. A_{Blank1} , and A_{Blank2} were defined as the absorbance of DDD water and sample without alpha-amylase enzyme.

- In vitro Assay for Measuring the Inhibition of Alpha-Glucosidase Enzyme

Alpha-glucosidase activity was determined by measuring the rate of the release of *p*-nitrophenol from 4-Nitrophenyl-alpha-D-glucopyranoside (PNPG) using colorimetric method that was adapted from Si *et al.* [21]. Briefly, the mixture consisted of 25 μ l of 3 mM L-glutathione (GSH), 250 μ l of 0.067 M potassium phosphate buffer (PPB; pH 6.8), and 25 μ l of 0.3 U/ml of alpha-glucosidase enzyme was added by 100 μ l of the rambutan rind extract, tannin, or acarbose dissolved in DDD water to give concentrations ranging from 0.15625 to 20 mg/ml, 1.25 to 20 mg/ml, and 10 mg/ml, respectively. The mixture was incubated in water bath at 37 °C for 10 min. After that, 25 μ l of 10 mM PNPG was added and incubated at 37 °C for 10 min. The reaction was stopped by adding 400 μ l of 0.1 M sodium carbonate solution. The absorbance of the assay mixture was measured by Benchmark Plus Microplate Spectrophotometer at 400 nm. All determinations were performed in triplicate. The inhibition percentage of alpha-glucosidase enzyme was assessed by following formula:

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

$$\Delta A_{\text{Control}} = A_{\text{Test1}} - A_{\text{Blank1}}$$

$$\Delta A_{\text{Sample}} = A_{\text{Test2}} - A_{\text{Blank2}}$$

Where: A_{Test1} and A_{Test2} were defined as the absorbance of DDD water and sample with alpha-glucosidase enzyme. A_{Blank1} , and A_{Blank2} were defined as the absorbance of DDD water and sample without alpha-glucosidase enzyme.

III. RESULTS

- Phenolic Content Analysis and Antioxidant Activity of the Rambutan Rind Extract

The rind of rambutan extracted by 85% hydroethanolic had a yield of 18.05% and total phenolic content of 416.18 ± 0.01 mg gallic acid/g dry extract. The rambutan rind extract exhibited a dose dependent inhibition of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) activity, with an IC_{50} value of 0.288 ± 0.04 mg/ml extract.

- Alpha-Amylase Enzyme Inhibitory Activity of the Rambutan Rind Extract and Tannin

Over the observed concentration range, dose dependent inhibition of the rambutan rind extract, but not tannin, on alpha-amylase enzyme activity was demonstrated as shown in Fig. 1 and 2. The maximum percentage inhibition on alpha-amylase enzyme activity of the rambutan rind extract and tannin were obtained at the concentration of 2.50 mg/ml (Fig. 1 and 2). At the same concentration (10 mg/ml), acarbose exhibited the highest inhibitory activity on alpha-amylase compared to the rambutan rind extract and tannin (Fig. 1 and 2).

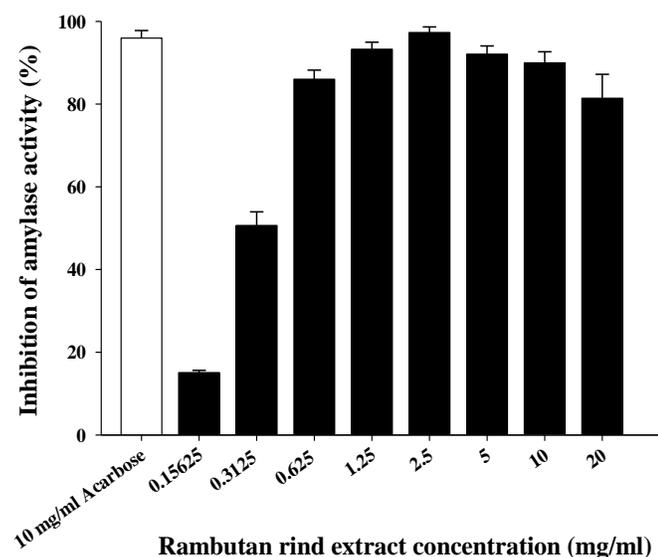


Fig. 1 Inhibitory effects of various concentrations of the rambutan rind extract and acarbose on alpha-amylase enzyme activity *in vitro*. Values are expressed as mean \pm S.E.M. of triplicate experiments.

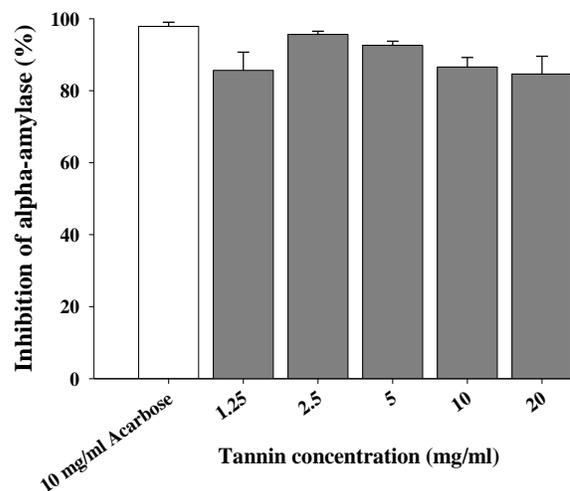


Fig. 2 Inhibitory effects of various concentrations of tannin and acarbose on alpha-amylase enzyme activity *in vitro*. Values are expressed as mean \pm S.E.M. of triplicate experiments.

- Alpha-Glucosidase Enzyme Inhibitory Activity of the Rambutan Rind Extract and Tannin

Dose dependent inhibition on alpha-glucosidase enzyme activity was observed at 0.15625, 0.3125, 0.625, 1.25, and 2.5 mg/ml of the rambutan rind extract and 1.25, 2.5, and 5 mg/ml of tannin. At the doses of 5, 10, and 20 mg/ml of the rambutan rind extract and 10, and 20 mg/ml of tannin, alpha-glucosidase enzyme inhibitory activity was slightly decreased (Fig. 3 and 4). The maximum percentage inhibition on alpha-glucosidase enzyme activity of the rambutan rind extract and tannin were obtained at the concentrations of 2.50 and 5 mg/ml, respectively. At the same concentrations (10 mg/ml), acarbose exhibited weaker inhibitory activity on alpha-glucosidase than the rambutan rind extract and tannin.

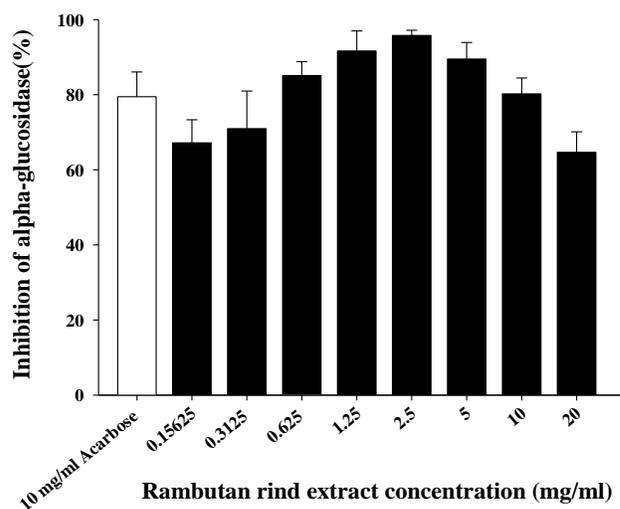


Fig. 3 Inhibitory effects of various concentrations of the rambutan rind extract and acarbose on alpha-glucosidase enzyme activity *in vitro*.

vitro. Values are expressed as mean \pm S.E.M. of triplicate experiments.

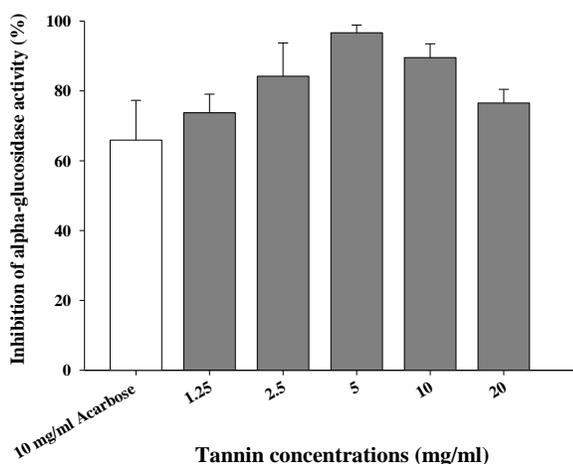


Fig. 4 Inhibitory effects of various concentrations of tannin and acarbose on alpha-glucosidase enzyme activity *in vitro*. Values are expressed as mean \pm S.E.M. of triplicate experiments.

IV. DISCUSSION AND CONCLUSION

The percentage yield of the rambutan rind extract obtained from this study was 18.05% which is similar to that found in previous study by Khonkarn *et al.*, Palanisamy *et al.* [15]–[17]. Ethanolic extract of the rambutan rind produced significant antioxidant activity, indicating that the rambutan rind extract exhibited a potential free radical scavenging activity [10]–[11]. This can be attributed to the total phenolic content present in the rambutan rind extract. Total phenolic content is very important plant constituents as their hydroxyl groups confer scavenging ability [22]. They are known as powerful chain breaking antioxidants. Thus, total phenolic content found in the rambutan rind extract tends to correspond with free radical scavenging activity [23]–[24]. Tannins are potent polyphenolic antioxidants found in the rambutan rind extract. Wongsiri *et al.* [25] revealed that the rambutan rind extracted by using water extraction had tannin content (21.18%). Tannin might be one of bioactive compounds found in the rambutan rind extract that responsible for anti-diabetic effect of the rambutan rind extract. Therefore, tannin was selected to be tested *in vitro*.

The results confirmed that the rambutan rind extract contains alpha-amylase and alpha-glucosidase inhibitors. The rambutan rind extract and tannin showed *in vitro* hypoglycemic activity. The rambutan rind extract and tannin inhibited alpha-amylase and alpha-glucosidase enzymes activities. Similar findings were observed by Palanisamy *et al.* [16]–[17], total phenolic content and geraniin from the rambutan rind extract were reported to have alpha-amylase and alpha-glucosidase enzymes inhibitory activities. Thus, the rambutan rind extract showed potentially promising effects for reduce blood glucose by blocking the digestion and absorption of dietary carbohydrates.

In conclusion, the present findings suggested that the rambutan rind extract and tannin displayed beneficial effects in the prevention and treatment of type 2 diabetes mellitus, possibly by inhibiting alpha-amylase and alpha-glucosidase activities. Further investigation is needed to clarify the effect of the rambutan rind extract and tannin on reduction of postprandial hyperglycemia *in vivo*.

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