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Mechanistic Studies on The Effect of *Nephelium lappaceum* Seed Powder on *In vitro* Glucose Uptake by *Saccharomyces cerevisiae*

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ABSTRACT

We proposed to elucidate the antidiabetic potential of *Nephelium lappaceum* (Rambutan) seed powder (peeled and unpeeled) by using in vitro bioassay. In this study, we investigated the antidiabetic potential of rambutan seed powder using in vitro glucose uptake mechanism by Saccharomyces cerevisiae (yeast). Rambutan seed powder (unpeeled) at a test concentration of 25 mg/mL was remarkable to enhance glucose uptake by yeast cells. In summary, rambutan seed powder found to possesses in vitro yeast glucose uptake enhancing potential.

Keywords: Antidiabetic potential; Glucose uptake by yeast cells; *Nephelium lappaceum*; Rambutan

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INTRODUCTION

Glucose is a signaling molecule that regulates various physiological processes; impaired regulation of blood glucose levels causes diabetes[1]. Diabetes is a chronic global health problem causing significant mortality and morbidity. World Health Organisation (WHO)predicts that diabetes (type 1 and type 2) will be the 7thmajorcause of death in the year 2030. Over the recent past two decades it has been understandable that the incidence of type 2 diabetes is rapidly increasing. Type 2 diabetes associated complications such as obesity, microvascular ischemia and inflammation includes 90% of the individuals triggered to diabetes every wherein the biosphere, and is basically the consequence of superfluous body weight and irregular physical activity. The global pathogenesis of type 1 and type 2 diabetes are occurring very fast[2]. If not, proper action is taken, by the year 2030, it is projected that there will be a minimum of 350 million people worldwide with diabetes. Type 2 diabetes is earlier termed as non-insulin-dependent or adult-onset diabetes, results from the body's unproductive use of insulin hormone. It is well-known fact that insulin is an endocrine hormone and powerful simulator of glucose transportation in skeletal muscle. In individuals with type 2 diabetes, insulin-mediated glucose uptake in skeletal muscle is decreased and this has led to develop ethanomedicine to improve clinical outcomes. In addition, the glucose sensing mechanism in the Saccharomyces cerevisiae(yeast) represents an important bioassay model for understanding glucose uptake and metabolism[3]. It expresses different isoforms of glucose transporters such as Hxt1-4, Hxt6, and Hxt7 respectively with different glucose uptake affinities[4]. The yeast glucose transporters transport glucose in a similar type of mechanism as the transporters (Glut) that transport glucose in humans[5].

Rambutan is a tropical tree cultivated mainly in Malaysia, Thailand and Indonesia[6]. The plant traditionally used for its anticancer[7], antihyperglycemic[8], antioxidant[9], antiviral[10], antimicrobial[11] and antiobesity[12] pharmacodynamic properties. Recent phytochemical studies on various parts of rambutan have led to the identification of polyphenols, monoterpene lactones and volatile compounds[13,14].A limited number of studies have been performed on bioactive potential of the rambutan seeds. Our study for the first time reveals the effect of rambutan seed powder (peeled and unpeeled) on yeast glucose uptake mechanism.

MATERIALS AND METHODS

Chemicals

Glucose, double-distilled water (DDW), dimethylsulfoxide (DMSO),Metformin HCl, *Saccharomyces cerevisiae* (baker's yeast),and concentrated sulfuric acid, all the chemicals were purchased from Sigma-Aldrich, Malaysia.

Instrumentation

Domestic blender, sieve shaker machine, electronic balance, beakers, funnels, test tubes, vortex mixer, centrifuge, micro pipette, incubator, quartz cuvettes, UV-double beam spectrophotometer (Jasco V-630).

Plant Materials

Rambutan fruits were purchased on 16th July 2016 from a local market in Selangor and taxonomy was authenticated by the science officer Dr. Richard Chung Cheng Kong, Forest Research Institute Malaysia (FRIM), Malaysia (Voucher Specimen Ref: FRIM700-1/1/1Klt.2(2)).

Preparation of rambutan seed powder

Rambutan seeds were separated from fruits in peeled and unpeeled form, dried, finely ground and sieved using sieve shaker machine. The solution of rambutan seed powder was made with DMSO at various concentrations (5-25 mg/mL) to make up the final volume of 5 mL. Subsequently, 0.5 mL of each concentration was used in the analysis.

***In vitro* glucose uptake by yeast cells bioassay**

Yeast (Saccharomyces cerevisiae) cells were prepared as per the method reported by Cirilloet.al.[15]. Commercially available baker's yeast (Saccharomyces cerevisiae) was washed repetitively by centrifugation (4200 rpm/min, 5 min) in distilled water till the supernatant liquids were clear and a 10% (v/v) suspension was prepared in distilled water. Different DMSO soluble concentrations of the rambutan seed powder such as 5, 10, 15, 20, and 25 mg/mL respectively were added to the 1 mL of glucose solution (5 mg/mL) and incubated all at once for 10 min at 37 °C. The reaction was started by adding 100 µL of yeast suspension, vortexed, further kept incubated at 37 °C for 60 min. Subsequently after 60 min, all the tubes were centrifuged (3800 rpm/min, 5 min) and the concentration of glucose was estimated in the supernatant (Table 1). The percent enhancement in glucose uptake by yeast cells was calculated by using UV-visible method based on the absorbance maximum of the control reaction that containing all reagents except the test sample and correspondingly by test sample (Table 2).

$$\% \text{ Glucose uptake by yeast cells} = \frac{\text{Absorbance } (\lambda_{max}) \text{ of control} - \text{Absorbance } (\lambda_{max}) \text{ of test}}{\text{Absorbance } (\lambda_{max}) \text{ of control}} \times 100$$

Sulfuric acid (H_2SO_4)-UV-visible method for glucose estimation

Glucose estimation was done by modification of sulfuric acid (H_2SO_4)-UV-visible method as described by Berhe et al., 2013[16]. A 1 mL of standard glucose solutions 1, 2, 3, 4 and 5 mg/mL was quickly mixed with 1 mL of concentrated sulfuric acid (conc. H_2SO_4) in a test tube and vortexed for 10 sec. The temperature (^oC) of the sample reaction mixture was raised instantaneously within 15sec after addition H_2SO_4. Then the solution was added with 2 mL of water and cooled for 10 min and brought it back to the room temperature. Lastly, UV-visible light absorption at wavelength of 315 nm was recorded using UV-visible double beam spectrophotometer (Jasco V-630). The glucose concentration of test solutions were estimated from a standard curve prepared with standard glucose solutions which were prepared using dilution method. The calibration curve for glucose estimations was plotted with a correlation coefficient (r2) of 0.990, indicating acceptable precision and accuracy of the method for the estimation of glucose (Table 2, Figure 1).

Statistical analysis

Statistical analysis has been carried out using statistical software GraphPad Prism v 5.0. All experiments were performed in triplicates (n=3) and the numerical results were obtained as mean±SEM. Group differences were determined using ANOVA (one-way analysis of variance); a statistical value of $P<0.05$ was taken as significant.

RESULTS

In vitro glucose uptake by yeast cells

Rambutan seed powder (peeled and unpeeled) was studied for in vitro glucose uptake by yeast (Saccharomyces cerevisiae) cells bioassay (Table 1). Results obtained from the study demonstrate that seed powder (peeled and unpeeled) enhanced glucose uptake by yeast (Saccharomyces cerevisiae) cells and in test concentration dependent way (Figure 2). Among the tested concentrations (5-25 mg/mL) of seed powders (peeled and unpeeled), unpeeled seed powder was exhibited slightly high percentage of uptake of glucose by yeast (Saccharomyces cerevisiae) cells at all tested concentrations (5-25 mg/mL). At maximum concentration i.e. at 25 mg/mL of each of peeled and unpeeled seed powder produced percentage enhancement of glucose uptake at 71.32 ± 0.05 and 78.01 ± 0.44 respectively. The observed data of seed powder was keeping with the good agreement in comparison with the concentration dependent activity exhibited by the positive standard Metformin HCl, but not at identical test concentrations (1-5 mg/mL).

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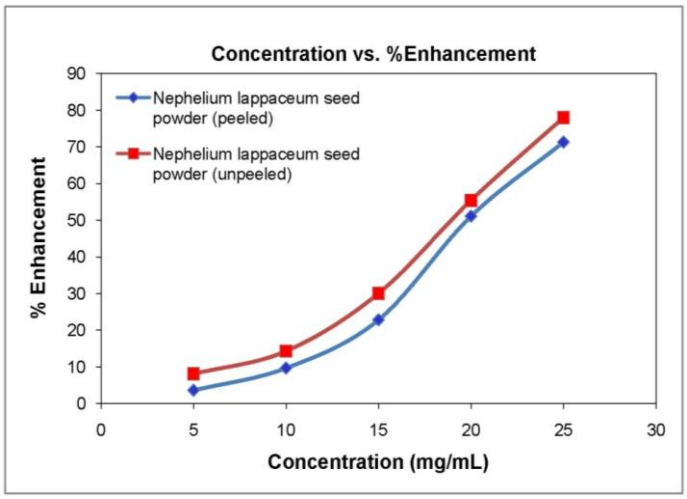
Table 1: Effect of *Nephelium lappaceum* (Rambutan) seed powder (peeled and unpeeled) against *in vitro* glucose uptake by yeast (*Saccharomyces cerevisiae*) cells

S.No	Test / Standard	Concentration (mg/mL)	Absorbance (λ_{max}) at 315 nm	% Enhancement*
1.	Control	-	0.6104± 0.01	0.00 ± 0.00
2.	*Nephelium lappaceum* seed powder (peeled)	5	0.5881 ± 0.03	3.64 ± 0.17**
		10	0.5511 ± 0.01	9.71± 0.01**
		15	0.4708 ± 0.01	22.87 ± 0.07
		20	0.2983 ± 0.01	51.12 ± 0.33
		25	0.1750 ± 0.05	71.32 ± 0.05
3.	*Nephelium lappaceum* seed powder (unpeeled)	5	0.5608 ± 0.03	8.12 ± 0.33**
		10	0.5229 ± 0.03	14.33 ± 0.02
		15	0.4268 ± 0.01	30.07 ± 0.12
		20	0.2719 ± 0.01	55.44 ± 0.63
		25	0.1342 ± 0.01	78.01 ± 0.44
4.	Metformin HCl (Standard)	1	0.5428 ± 0.03	11.07 ± 0.12
		2	0.4184 ± 0.03	31.44 ± 0.74
		3	0.3413 ± 0.01	44.07 ± 0.41
		4	0.2585 ± 0.01	57.64 ± 0.14
		5	0.2093 ± 0.02	65.71 ± 0.48

P<0.05, * Results were expressed as mean±SEM (n=3), **Not significant (<10%)

Figure 2: Effect of *Nephelium lappaceum* (Rambutan) seed powder (peeled and unpeeled) against *in vitro* glucose uptake by yeast cells



Sulfuric acid (H_2SO_4)-UV-visible method for glucose estimation

The estimation of glucose in supernatant by using UV-visible method was calculated based on the absorbance at wavelength of 315 nm. The calibration plots were shown in (Table 2, Figure 1).

Table 2: Standard plots for estimation of glucose using sulfuric acid-UV method at 315 nm.

S. No	Concentration of glucose standard solution	Absorbance response (λ_{max}) at 315 nm*
1.	Blank	0.2653 ± 0.11
2.	Glucose standard 1 (mg/mL)	0.3773 ± 0.14
3.	Glucose standard 2 (mg/mL)	0.5306 ± 0.43
4.	Glucose standard 3 (mg/mL)	0.6139 ± 0.24
5.	Glucose standard 4 (mg/mL)	0.7991 ± 0.51
6.	Glucose standard 5 (mg/mL)	0.9185 ± 0.74

*Results were expressed as mean±SEM (n=3)

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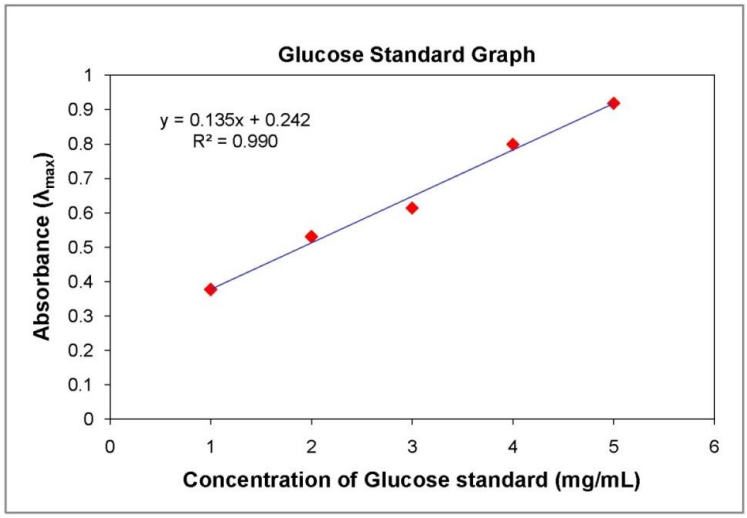
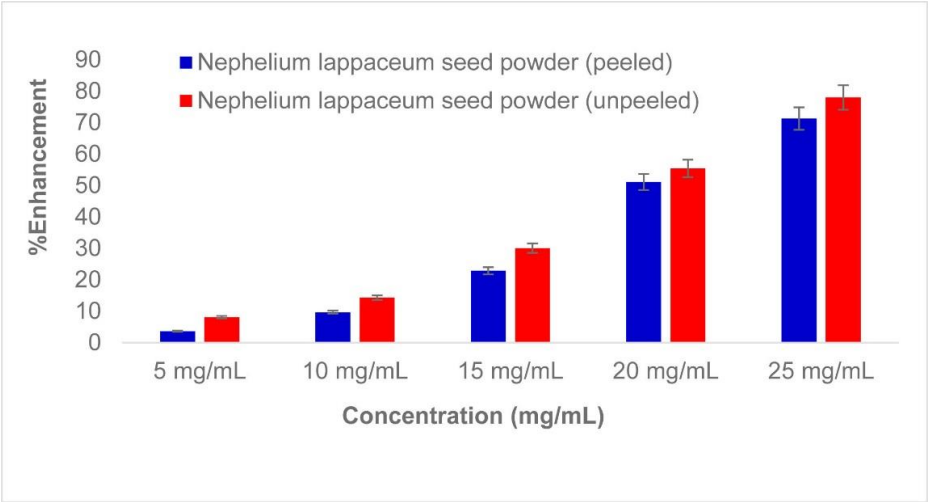
Figure 1: Standard graph for estimation of glucose using sulfuric acid-UV method at 315 nm.



DISCUSSION

In vitro glucose uptake by yeast cells

Figure 3: Relative effect of *Nephelium lappaceum* (Rambutan) seed powder (peeled and unpeeled) against *in vitro* glucose uptake by yeast cells



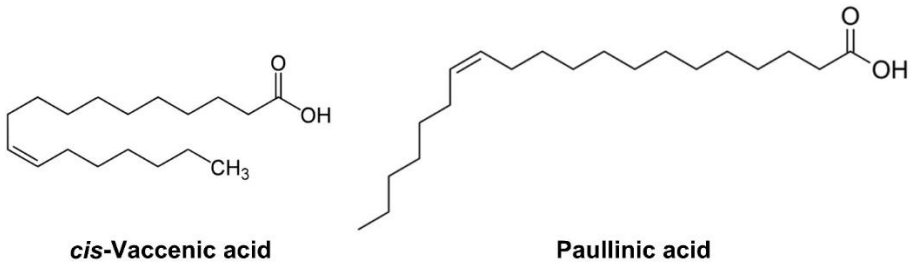
By comparing the results of rambutan (peeled and unpeeled) revealed that the percentage enhancement of glucose uptake was varied with different test concentrations ranging from (5-25 mg/mL) as shown in Figure 3. The unpeeled rambutan seed powder showed 3.64 ± 0.17% (5 mg/mL) and 9.71 ± 0.01% (10 mg/mL) enhancement of glucose uptake which was relatively less compared to unpeeled rambutan seed powder with 8.12 ± 0.33% and 14.33 ± 0.02% at similar test concentrations. Likewise, the relative comparison between other test concentrations such as 15, 20 and 25 mg/mL exhibited the predominance of unpeeled rambutan seed powder with percentages of enhancement of glucose uptake as 30.07 ± 0.12%, 55.44 ± 0.63% and 78.01 ± 0.44% against peeled rambutan seed powder with percentages 22.87 ± 0.07%, 51.12 ± 0.33% and 71.32 ± 0.05% respectively at identical test concentrations (15, 20 and 25 mg/mL) in each case. Similarly, Metformin HCl standard was used as positive control to the experiment, which was also been exhibited a concentration dependent percentage enhancement of glucose uptake (1-5 mg/mL). Our hypothesis was well supported and consistent with few other research findings with respect to the reported antidiabetic property of rambutan seeds, rambutan seed infusion has showed effect in reducing the body weight and elevated blood glucose levels of alloxan-induced diabetic mice at 3.12 g/kg bw which was comparatively achieved with clinically used drug glibenclamide 0.65 mg/kg bw[17]. Furthermore, the key mechanisms by which rambutan

seeds may affect the diabetic conditions are by enhancing free radical scavenging activity[18], increasing total superoxide dismutase (SOD) activity[19], inhibiting glucose-6-phosphate dehydrogenase (G6PDH) enzyme activity[20], inhibiting α-glucosidase enzyme activity and by decreasing triglyceride (TG) levels[21] which are possible due to phytochemical constituents in rambutan seeds[22].

Moreover, rambutan seed oil has been reported to contain composition of triglycerides (83%) and cyanolipids (17%) containing 1-cyano-2-hydroxymethylprop-1-en-3-ol diesters and type III cyanolipids. Oleic acid and arachidic acid were the two-major esterified fatty acyl chains found in both cyanolipids and triglycerides [23].

The eicosenoic acids (Figure 4)like cis –vaccenic and paullinic acidwere chiefly present in larger amounts in the fractionated cyanolipid of the oil which were practically reported to be non-toxic[24].In summary, this research enabled us to extract significant facts about antidiabetic potential of rambutan seed powders (peeled and unpeeled) and paved the way towards the scope of developing polyhedral formulations by including rambutan seed powder as one of the active ingredients.

Figure 4: Chemical structures of cis –vaccenic and paullinic acids.



***cis*-Vaccenic acid** **Paullinic acid**

CONCLUSION

Based on the present study it can be concluded that the seed powder of rambutan possess in vitro yeast glucose uptake enhancing activity which might be due to the presence of bioactive phytochemical constituents. However further investigations are essential to identify the underlying mechanism of action for individual phytochemical constituent. In addition, this research has provided a mechanistic insight of rambutan seed powder for management of type 2 diabetes.

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