

**SCREENING OF PHYTOCHEMICALS AND DETERMINATION OF ANTIHYPERGLYCAEMIC ACTIVITY ON ETHANOLIC EXTRACT OF FRESH FRUIT OF NEPHELIUM LAPPACEUM L. ON FEMALE SPRAGUE – DAWLEY RATS**

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**ABSTRACT**

Screening of phytochemicals and determination of antihyperglycaemic activity of ethanolic extracts of *Nephelium lappaceum* L (EENL) fresh fruit on female Sprague-Dawley rats. The extraction for *Nephelium lappaceum* was done by using 96% ethanol solvent and its pharmacological action was screened. Adult female healthy Sprague Dawley (SD) rats were used for the testing determination of the antidiabetic activity of EENL. In the screening, the rats were divided into 3 different groups with 3 animals each and tagged with standard control, 1mL and 2mL PO of 4000mg/kg EENL. Apart from the standard control group, the rest of the SD rats were given with a freshly prepare single dose intraperitoneal infection of 55mg/kg streptozotocin (STZ) to induce diabetes mellitus. Follow on the rats with diabetes mellitus was given with EENL for consecutives 21 days through oral route feeding. The blood sample was collected on 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day via caudal vein. From the study, EENL shows it has the capabilities to reduce glucose level in the rats. However the different dosing for the rats did not show a significant change in the final result.

**KEYWORDS:** Diabetes mellitus, Streptozotocin, *Nephelium lappaceum*, antihyperglycaemic.**INTRODUCTION**

*Nephelium lappaceum* L.), family of Sapindaceae is one of the famous tropical fruit in Asia. It is well known for its tangerine hairy skin either in red, orange or yellow colour with ovoid shape.<sup>[34]</sup> The inner part of rambutan consists two part, the edible fruit pulp and oblong rough surface seeds which covered by the fruit pulp. Edible fruit pulp possess enormous of sugars, organic acids and ascorbic acid.<sup>[35]</sup> Besides that, of the fruit pulp is having slightly shining and translucent white surface. Flavor of the pulp is almost similar to *Litchi chinensis* (Lychee), however it has lesser aromatic and juicy but it is somewhat firmer when squish it softly.<sup>[36]</sup> The constituent of rambutan fruit is 27.4% of weight, 13.2% peel, 11.7% pulp, 2.53% seed and 1.60% embryo.<sup>[36]</sup> Rambutan required a warm, humid and low evaporation rates with high amount of rainfall to growth. Thus, the fruits particularly mass-produce specifically in certain country around Southeast Asia. This country includes Malaysia, Indonesia, and Thailand.

**Figure 1: Rambutan peel and pulp.**

There are approximately 20 species of rambutan species was identify within Malaysia out of 10 species can be found in the natural. This species included *N.aculeatum*, *N.compressum*, *N.costatum*, *N.daedaleum*, *N.hamulatum*, *N.havilandii*, *N.macrophyllum*, *N.meduseum*, *N.papillatum* and *N.reticulatum*. However, the only species that will be more focusing will be the *Nephelium lappaceum*. Other rambutans such as pulasan (*N.rambutan-ake*) and kalambuko (*N.cuspidatum*) are used to cultivate as fruits to supply Malaysian. Species like kalas (*N.daedaleum*), gerringgong (*N.lauriman*), buah sungkit (*N.maingayl*) and mertapang (*N.melanomiscum*) produce edible sour like fruit.<sup>[38,39]</sup>

**Table 1: Taxonomical classification of rambutan.**

<b>Domain</b>	<b>Eukarya</b>
<b>Kingdom</b>	Plantae
<b>Subkingdom</b>	Tracheobionta
<b>Division</b>	Tracheophyta
<b>Class</b>	Magnoliopsida
<b>Subclass</b>	Rosidae
<b>Order</b>	Sapindales
<b>Family</b>	Sapindaceae
<b>Genus</b>	Nephelium
<b>Species</b>	Nephelium lappaceum

**Figure 2: Rambutan raw sample.****Figure 3: Flower of rambutan.****Figure 4: Rambutan pulp.****Figure 5: Rambutan Seed.**

Multiple phytochemical studies had carried out to identify the bioactive constituents possessed by the rambutan. Several chemicals and reagent were used such as 1% ferric chloride, Mayer's reagent, Liebermann Burchard reagent, distilled water etc. The screening of the fruits provided the bioactive compounds of alkaloids, glycosides, reducing sugar and flavonoids. A research study of Qingyu Ma *et al* reported phenolic extract of *Nephelium lappaceum* extract demonstrated high antioxidant and anti-glycaemic activities in vitro and vivo studies. The studies tested on mouse with high fat diet and proven anti-diabetic effect had improve the diabetic disease significantly and increases body weight.<sup>[40]</sup> A research study done by Uma Palarisamy *et al* reported *Nephelium lappaceum* effective in inhibiting enzyme (glucosidase and amylase) responsible for the hydrolyzing carbohydrates than medication (acarbose) use. Besides that, inhibiting enzyme, the active constituents (geranin) still possess the ability to inhibit key enzyme in the polyol pathway, aldol reductase and prevent the formation of advanced glycation end products (AGE).<sup>[41]</sup>

A research study carried out by Shonia Subramaniam *et al* reported ethanolic *Nephelium lappaceum* was prepared and standardized by using HPLC on male Sprague-Dawley rats that fed with high fat diet and induced with diabetes with streptozotoin. The diabetic rats treated with 500 and 2000mg for 28 days of *Nephelium lappaceum* and the reduction of glucose and improved of insulin have shown. Besides that, 2000mg of *Nephelium lappaceum* treated rats has shown a healthy pancreas morphology when compared to metformin treated group.<sup>[42]</sup> Extraction of *Nephelium lappaceum* shows the antibacterial activity against *Staphylococcus aureus* regardless what kind of extraction solvent was used. Different solvent extraction provide different level of antibacterial activity such as the extraction of rambutan peels is through ether, methanol and aqueous extract are having the activity against *Vibrio cholera*, *Enterococcus faecalis*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.<sup>[44]</sup>

Screen the phytochemical and determine antihyperglycaemic activity of *Nephelium lappaceum* L



fresh fruit. Secondly, the antihyperglycaemic activity of *Nephelium lappaceum* L fresh fruit is used to test on female Sprague-Dawley rats.

## METHODOLOGY AND EXPERIMENTAL

### Materials and Apparatus

500mg Carboxymethyl cellulose (CMC) in 500 of normal saline, Ethanol 96%, Streptozotocin (STZ), Concentrated Sulphuric acid (96%), Concentrated Hydrochloric acid (37%), Sodium Hydroxide solution (10%), Dilute Sulphuric acid (2%), 1% ferric chloride, Diluted hydrochloric acid (10%), Ammonia solution (10%), Fehling's solution A, Fehling's solution B, Lead acetate solution, Acetic acid, Mayer's reagent, Wagner's reagent, Molisch's reagent (solution of alpha-naphthol in 95% ethanol), Barfoed's reagent (solution of cupric acetate and acetic acid).

Ethanol, 0.75% sodium bicarbonate solution, gallic acid, 1% Folin-Ciocalteu reagent, Animal cages, animal water feeding bottle, and oral feeding tubes.

All biological wastes were disposed through AIMST University waste management system.

### Animals

Adult and healthy female Sprague Dawley (SD) rats were used. They were weighing around  $200 \pm 50$ g, which obtained from the Central Animal house, AIMST University, Malaysia. All the SD rats were placed and maintained in a large and sufficient movement space of poly acrylic cages (3 cages) in a normal room temperature  $24^{\circ}\text{C}$  with 12 hour light and 12 hour night cycle. Sufficient food and filtered water were given throughout the experiment. The approval of Human and Animal Ethics was obtained before the study was carried

out. The research was conducted according to the Animal Research Review Panel Guidelines.

### Methods

*Nephelium lappaceum* L fresh fruit was prepared through buying with a vendor in Gurun, Kedah. The peel and seeds of the rambutan was taken off and then the rambutan fruits was put into a 3L round bottom conical flask. Before the extraction process, the rambutan was weighted on the weighting balance. The initial weight of rambutan with basket was measured. The weight of the basket is 270.70g while the weight of rambutan with basket is 1767.33g, subtracted out the weight of the basket, the actual weight of rambutan fruits is  $1496.63 \pm 20$ g. Thus, the rambutan fruit can be considered as 1.5kg. Then the rambutan fruits was separated into smaller pieces and added into 3L of round bottom flask as shown in the figure 2. 1.5L of 95% ethanol was measured and added into the 3L round bottom flask through a funnel to prevent spillage. Maceration technique was implemented on the extraction process of rambutan fruits. The extraction process was carried out at room temperature. Rambutan fruits were allowed to immerse in the 95% ethanol with an airtight mechanism applies onto the 3L round bottom flask. This technique enables the active ingredients slowly diffuse out from the rambutan fruits. The rambutan fruit was allowed to be in maceration process for 10 days at room temperature with air tight mechanism. After 10 days, the extracted content was filtered with muslin cloth and filter funnel into a new 3L of round bottom flask, leftover fruits was squeezed out the remaining mixture by using hand with glove on.

### Phytochemical screening

2% of extract was prepared by dissolving 1.0g of extract in 50mL of distilled water.

**Table 2: Preliminary qualitative analysis results of phenolic compounds isolated from *Nephelium lappaceum* L fruit.**

Reagents	Detection results	Indications	Conclusion
Mayer	+	Formation of cream or pale yellow precipitate	Presence of alkaloids
Wagner	+	Formation of reddish brown precipitate	Presence of alkaloids
Fehling	+	Formation of brick red precipitate	Presence of reducing sugar and glycosides
Barfoed	+	Formation of reddish precipitate	Presence of reducing sugar
Molisch	+	Formation of violet ring	Presence of carbohydrate
Lead acetate	+	Formation of yellow color precipitate	Presence of flavonoids
1% ferric chloride	-	No presence of dark green solution	No presence of tannins
10% ammonia solution	-	No formation of rose pink color	No presence of anthraquinones
Mix with water	-	No formation of small bubbles	No presence of saponin
Salkowski	-	No discoloration of reddish brown	No presence of terpenoids
Liebermann Burchard	-	No color changes occur	No presence of terpenoids

+ : positive - : negative

**Table 3: Phytochemical analysis of *Nephelium lappaceum* fruits ethanol extract.**

Sample	Ethanolic method
Alkaloids	+
Glycosides	+
Reducing sugar	+
Flavonoids	+
Tannins	-
Anthraquinones	-
Saponin	-
Terpenoids	-

**Antihyperglycaemic activity of ethanol extract of *Nephelium lappaceum* L**

Female Sprague Dawley rats (SD rats) were divided into 3 different groups with 3 animals in 2 groups and 2 animals in 1 group as following:

**Group 1:** Standard group

**Group 2:** Diabetic animals treated with 2mL EENL (4000mg/kg)

**Group 3:** Diabetic animals treated with 1mL EENL (4000mg/kg)

The rats were first being fasted with only water overnight. Follow on next day, the rats was induced with diabetes mellitus by administering them with freshly prepared 55mg/kg/mL of streptozotocin (STZ) via intraperitoneal injection. STZ intraperitoneal injection

preparation was prepared by dissolving 110mg of STZ in 2mL of distilled water at the neutral pH. After the preparation, the injection was immediately administered to the SD rats as the stability of the injection is approximately 30 to 60 minutes.<sup>[49]</sup> Then the rats were given glucose solution 5% w/v (2mL/kg BW) for 24 hours to induce diabetes mellitus and to prevent hypoglycaemic lead to fatal. The diabetes mellitus was confirmed after 48 hours of administration of intraperitoneal injection by measuring their fasting glucose level. Blood sample collection was via caudal vein. Rats with fasting blood glucose more than 7mmol/dL were used as experiment subject.

Group 1 (standard group) was given only normal saline while rats in group 2 and group 3 were receiving ethanol extract mixed with 5% CMC (carboxymethyl cellulose) to get a concentration of 4000mg/kg. However, the dose given to both rats in group are different, the rats in group 2 is receiving 2mL while group 3 is receiving 1mL.

All groups of rats were fed once daily through oral gavage for 21 consecutive days. Blood sample were collected through caudal vein on 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of the experiment and immediately used on blood glucose meter to determine the blood glucose level.

**Antidiabetic effect of EENL on SD rats****Table 4: Result of Group 3 SD rats 1mL of (40mg/mL).**

<b>Group 1 (Control)</b>					
No. of Rats	Day 1	Day 7	Day 14	Day 21	
1	4.1	4.3	4.2	4.3	
2	4.5	4.4	4.4	4.5	
Mean	4.3	4.35	4.3	4.4	
<b>Table 5.2.1: Result of Group 1 SD rats.</b>					
<b>Group 2 (rats feeding with 2mL (40mg/mL) of extract)</b>					
No. of Rats	Day 1	Day 7	Day 14	Day 21	
1	7.3	4.4	4.3	3.9	
2	7.3	4.8	4.6	5.2	
3	7.8	4.6	4.4	5.9	
Mean	7.4667	4.6000	4.4333	5.0000	
<b>Table 5.2.2: Result of Group 2 SD rats 2mL of (40mg/mL).</b>					
<b>Group 3 (rats feeding with 1mL (40mg/mL) of extract)</b>					
No. of Rats	Day 1	Day 7	Day 14	Day 21	
1	7.1	4.7	3.2	4.4	
2	9.2	5.3	5.9	5.0	
3	7.8	4.9	4.7	4.8	
Mean	8.0333	4.9667	4.6000	4.7333	

## DISCUSSION

Diabetes mellitus is a compilation of diverse diseases that differ in the sense of clinical presentation, epidemiology and etiological. However, with the presence of hyperglycaemia and glucose intolerance, this is either be insulin deficiency or insufficient insulin action or both. Some synthetic drugs such as biguanides, sulfonylureas, meglitinides, thiazolidinediones,  $\alpha$ -Glucosidase Inhibitors, Incretin Mimetics and Incretin Enhancers Drugs are usually being the favour to treat diabetes mellitus. Nevertheless, these kind of synthetic drugs possess several adverse effect that may cause more complication towards to the patient. For example, the most common side effect cause by hypoglycaemic medication is weight gain, sometime possible of hypoglycaemic incident shows in certain class of hypoglycaemic medication.

Thus, this is where natural agents come in and play an important role in treating diabetic mellitus due to it has a better potential of pharmacological action. Besides that, the development of new hypoglycaemic agent via natural agents might have the possibilities in reducing the side effect of synthetic drug. *Nephelium lappaceum* has selected for this study because some studies proven that are a good hypoglycaemic activity. It is from Sapindaceae family and can be widely found in Asia. *Nephelium lappaceum* had reported with several useful pharmacological actions such as hypoglycaemic, antibacterial, cytotoxic activities, reduce hypercholesterol and etc.

The evaluation of anti-hyperglycaemic effect by *Nephelium lappaceum* has conducted by using ethanolic form of the extraction and given to induced diabetic mellitus in Sprague Dawley strain of female rats. The results were obtain via blood collection on caudal vein and apply on a glucose meter. The diabetes group of rats having higher glucose reading level than the control group, this indicate the rats is having diabetes mellitus. On the other hand, the control group of rats is having normal glucose level.

The result obtained from this study showed that EENL has the ability of reduce glucose level in the rats when compare to control group. This indicates that EENL can reduce glucose level which induced by STZ. Base on the tables above Table 5.2.2 and 5.2.3, there is no significant reduce of glucose level when given in different doses. However, this dosing study should be carry out more to determine the actual dosing and the difference dosing affect the reading of glucose level.

## CONCLUSION

Phytochemical screening of ethanolic extract of *Nephelium lappaceum* L via maceration method and determined the presence of alkaloids, glycosides, reducing sugar, and flavonoids. In contrast, it also revealed there is absence of tannins, anthraquinones,

saponins and terpenoids in ethanolic extract of *Nephelium lappaceum* pulp.

The antidiabetic activity of the ethanolic extract of *Nephelium lappaceum* extract was determined by introducing the SD rats diabetic mellitus with streptozotocin and treated with it. The antidiabetic activity was seen on the rats, the glucose level of the rats was reduced. However, there is no significant reduce of glucose level when given in different doses.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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This research paper has not been submitted anywhere else.

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