INVESTIGATION ON SOME BIOACTIVITIES AND THE NUTRIENTS OF *NEPHELIUM LAPPACEUM* L. (KYET-MAUK) SEEDS

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Abstract

Nephelium lappaceum L. (*Sapindaceae*) popularly known as Kyet-mauk in Myanmar which is one of the traditional medicinal plant. So, *N. lappaceum* (Kyet-mauk) seeds were chosen for this research work. The present research work was designated to evaluate the antioxidant, antidiabetic, antimicrobial and cytotoxic activities of *N. lappaceum* seed. The preliminary phytochemical investigation by test tube method revealed the presence of alkaloids, α–amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, tannins and terpenoids. Cyanogenic glycosides and steroids were not found in *N. lappaceum* seed. Investigation on nutrients (moisture, ash, fat, protein, fiber and carbohydrate) of *N. lappaceum* seed has been carried out by AOAC method. An energy value (Kyet-mauk) seed was found to be 430.07 kcal/100 g on the basis of dried sample. The mineral contents: K, Ca, Fe, Zn, Mn, Cu, and Rb were relatively observed by EDXRF spectroscopy. Among them, potassium content of the sample was the highest (49.528 %). In the screening of the antioxidant activity by DPPH method, IC_{50} values of ethanol and watery extracts were found to be 16.34 μg/mL and 17.31 μg/mL, respectively. *In vitro* α-amylase inhibitory activity of ethanol and watery extracts was investigated by using the starch-iodine method. The percent inhibition of α-amylase activity of ethanol extract (IC_{50} = 80.58 μg/mL) and watery extract (IC_{50} = 178.86 μg/mL) indicated the superiority of ethanol extract over watery extract. The total phenol contents (TPC) of ethanol and watery extracts of *N. lappaceum* seed were determined by FCR method. The TPC contents of ethanol and watery extracts were respectively observed to be (79.88 ± 2.86 μg GAE/mg) and (30.73 ± 7.65 μg GAE/mg). The antimicrobial activities of the various crude extracts (petether, acetyl acetate, ethanol and watery extracts) of *N. lappaceum* seed sample were determined against six strains of microorganisms by agar well diffusion method. The ethanol extract showed the antimicrobial activity with the inhibition zone diameters range 10 ~ 14 mm against six microorganisms such as *B. subtilis, S. aureus, P. aeruginosa, B. pumilus, C. albicans and E. coli*. The cytotoxicity of ethanol and watery extracts of *N. lappaceum* seed were evaluated by brine shrimp cytotoxicity bioassay. The LD_{50} values of ethanol and watery extracts were $LD_{50} >\geq 1000$ μg /mL.

Keywords: *Nephelium lappaceum* L., nutritional values, antioxidant activity, α-amylase inhibition activity, antimicrobial activity, cytotoxicity

Introduction

The term “medicinal plant” includes various types of plants used in herbalism ("herbology" or" herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses. Nowadays, herb refers to any part of the plantlike fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term “herb” was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities. Plants have been used for medicinal purposes. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used

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drugs for infectious diseases have led to increase emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. The use of herbal is independent of any age groups and the sexes. This is the reason why herbal treatment is growing in popularity across globe. These herbs that have medicinal quality provide rational means for the treatment of many internal diseases, which are otherwise considered difficult to cure. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values (Zahid , 2016 and Thomas *et al.*,1998). The use of herbal remedies is more prevalent in patients with chronic diseases such as cancer, diabetes, asthma and end-stage renal disease (WHO, 1991).

Description and Distribution of *Nephelium lappaceum* L.

N. lappaceum known as rambutan is one of variety tropical fruit which commonly consumed in south-east Asia (Figure 1). The rambutan is a medium-sized tropical tree in the family *Sapindaceae*. It is an evergreen tree growing to a height of 12–20 m. Rambutan tree loves the tropical climates, around 22-30 ºC and is sensitive to temperatures below 10 ºC. The rambutan is native to the Malay-Indonesian region and other tropical regions of Southeast Asia. Fruit is around to oval single seeded berry (3-6 cm long and 3-4 cm broad). The leathery skin is from green to red (rarely orange or yellow). The single seed is glossy brown 1–1.3 cm, with a white basal scar. Flowers are greenish white, fragrant, very small, without petals and borne on axillary panicles. Leaves are pinnate and elliptic (Suganthi and Josephine, 2016). Trees begin flowering from March to May and August to October. Fruits mature from 15 to 18 weeks after flowering. The rambutan fruit can be used in a wide range of products including beverages, dairy products, desserts, jams and gum. It is an important commercial crop in Asia, where the fruits are consumed fresh, canned in syrup, or processed, and appreciated for its refreshing flavor and exotic appearance. There is now been a steady increase in the production of rambutan canned in syrup with an increase in the commercial production of rambutan for canning purposes (Manaf *et al*., 2013).

Botanical Aspect of *Nephelium lappaceum* L. (Kyet-mauk)

Family	-	*Sapindaceae*
Genus	-	*Nephelium* L.
Species	-	*N.lappaceum*
Botanical name	-	*Nephelium lappaceum* L.
Myanmar name	-	Kyet-mauk
English name	-	Rambutan
Part used	-	Seeds

(a)

(b)

(c)

Figure 1 Photographs of (a) Plant (b) Fruit(c) Seeds of *Nephelium lappaceum* L. (Kyet-mauk)

Medicinal Uses and Chemical Constituents of *N. lappaceum* (Kyet mauk)

The rambutan is a rich source of vitamins and minerals that aids in losing weight, strengthening bones, and also offers anti-parasitic properties. The leaves can be juiced and used for a healthy scalp. The bark is known to cure sores. In addition, it also possesses biological activities such as antioxidant and antimicrobial. The rambutan seeds are useful to enhance skin texture and to against diabetes (Sukmandari *et al*., 2017).

A single light brown rambutan seed contains the saturated and unsaturated fatty acids. The arachidic acid is saturated fatty acid. The oleic acid, monounsaturated omega-9 fatty acid reduces blood pressure, increases good cholesterol and cuts the risk of developing ulcerative colitis. The arachidonic acid, polyunsaturated omega-6 fatty acid increases protein synthesis resulting in increased aerobic capacity and muscle hypertrophy (De *et al*., 2014 and Rajasekaram *et al*., 2013).

Rambutan seeds are not poisonous and contain carbohydrate, fats, proteins, which can meet the needs of the body of nutrients. There is now a convenient source of rambutan seeds for fat extraction. The fat has been used in cooking and the manufacture of soap. The fat contains oleic and arachidic acids as the dominant fatty acids. Rambutan seed fat also has the potential for conversion into a biodiesel or fuel extender because of its high cetane index (67.1). Rambutan roots, bark and leaves have various uses in medicine and in production of dye (Manaf *et al*., 2013). Rambutan has antioxidant activity and high phenolic content. Therefore, we conducted our research to evaluate antioxidant and hypoglycemic activity of rambutan's seed extract. The aim of the research here is to study on the Myanmar traditional herbs that play a very important role in the development of new drugs. The objective of this research is to find out the potential antioxidant, anti-diabetes and antimicrobial of herbal drugs.

Material and Methods

Sample Collection and Preparation of *N. lappaceum* (Kyet-mauk)

The *N. lappaceum* (Kyet-mauk) seeds were collected from Belin Township, Mon State. The sample was identified at Department of Botany, University of Yangon. The collected samples were washed, peeled leathery skin and edible skin then the seeds were cut into small pieces, dried at room temperature and ground into powder by motor. The sample was stored in air-tight container to prevent moisture changes and other contamination.

Preliminary Phytochemical Investigation of *N.lappaceum* (Kyet-mauk) Seeds

In order to find out the types of phyto-organic constituents such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, and terpenoids in the sample, preliminary phytochemical tests were carried out according to the appropriate methods. Various crude extracts (pet-ether, ethyl acetate and ethanol)of *N. lappaceum* were prepared for TLC screening, which were loaded on the precoated TLC silica gel plate and the chromatography was carried out by using an appropriate standard solvent system for *N. lappaceum* . The developed chromatograms were first inspected under UV-254 nm and 365 nm light and then sprayed with detecting reagents to classify the compounds present and their functional groups.

Qualitative Elemental Analysis by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry

About 1 g of dried sample was fabricated into the pellet. The sample was placed in the sample chamber of EDX-720 spectrometer that can measure the sixteen samples at the same time. The chamber was pumped up to vacuum. The pressure was about 88 pa and the detector temperature is about 170 °C. Therefore, liquid nitrogen needs to be added at the time of analysis. Rhodium target was used in (Shimadzu EDX-720) spectrometer Universities' Research Center, University of Yangon. Each sample was run for a counting time of about 100 seconds and the spectrum obtained was stored using EDX-720 software (Griken, 1993).

Determination of Nutrients of *N. lappaceum* (Kyet-mauk) Seeds

Nutritional values such as moisture content, ash content, fat content, fiber content, protein content, carbohydrate content and energy value of the selected sample were determined by AOAC method (AOAC, 2002).

Preparation of Ethanol and Watery Extracts from *N. lappaceum* (Kyet-mauk) Seeds

The dried powder sample (100 g) was percolated with 95% ethanol (500 mL) for one week and filtered. This procedure was repeated for three times. The combined filtrate containing plant constituents were evaporated under reduced pressure by means of a rotary evaporator. Consequently, 95% ethanol soluble extract was obtained. The 95 % ethanol extract was then partitioned with pet-ether (60-80 °C) (500 mL) by using separatory funnel. The pet-ether fraction was removed under reduced pressure in a rotary evaporator. The pet-ether extract was obtained. The defatted residue was then extracted 3 times with 95 % ethanol (500 mL) for one week by percolation. Removal of the solvent from combined ethanol fractions provided ethanol crude extract. Watery extract of three samples was prepared by boiling 100 g of sample with 500 mL of distilled water for 6 h and filtered. It was repeated three times and the filtrates were combined followed by heating on water bath and sand bath to give watery extract. Each extract was stored in refrigerator for screening of biological activities.

Determination of Antioxidant Activity of Ethanol and Watery Extracts of *N. lappaceum* (Kyet-mauk) Seeds by DPPH Free Radical Scavenging Assay

The free radical scavenging activity of crude extracts of *N. lappaceum* (Kyet-mauk) seeds was measured by using DPPH free radicals scavenging assay (Marinova and Batchvarov, 2011). DPPH radical scavenging activity of ethanol and watery extracts of Kyet-mauk seeds was determined by UV-visible spectrophotometer. The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol in the brown bottle. The blank solution was prepared by mixing the sample solution 1.5 mL with ethanol 1.5 mL.The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution with the concentrations of 40, 20, 10, 5, 25, 1.25 μg/mL. These bottles were incubated at room temperature and were shaken on shaker for 30 min. After 30 min, these solutions were measured at 517 nm and the percentage of radical scavenging activity (% RSA) was calculated by the following equation.

$$\% \text{ RSA} = [\{(A_{DPPH} - A_{sample}) - A_{blank}\} / A_{DPPH}] \times 100$$

Where, % RSA = % radical scavenging activity of test sample

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{sample} = absorbance of sample+ DPPH solution

A_{blank} = absorbance of sample + EtOH solution

The antioxidant power (IC_{50}) is expressed as the test substances concentration (μg/mL) that result in a 50 % reaction of initial absorbance of DPPH solution and that allows to determine the concentration. IC_{50} (50 % inhibition concentration) values were calculated by linear regressive excel program.

Determination of α-Amylase Enzyme Inhibition Activity of Ethanol and Watery Extracts of *N. lappaceum* (Kyet-mauk) Seeds and Standard Acarbose

Alpha-amylase activity can be measured for *in-vitro* by hydrolysis of starch in the presence of α-amylase enzyme. This process was quantified by using iodine, which gives blue colour with starch. The reduced intensity of blue colour indicates the enzyme-induced hydrolysis of starch into monosaccharide. If the substance/extract possesses α-amylase inhibitory activity, the intensity of blue color will be more. In other words, the intensity of blue colour in test sample is directly proportional to α-amylase inhibitor activity (Mandal and Reddy, 2016). Alpha-amylase activity was carried out by starch-iodine method. 10 μL of α-amylase solution (0.025 mg/mL) was mixed with 390 μL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) with different concentrations of extracts (400, 200, 100, 50, 25 and 12.5 μg/mL). After incubation at 37 °C for 10 min, 100 μL of starch solution (1 %) was added, and the mixture was re-incubated for 1 h. Next, 0.1 mL of 1 % iodine solution was added, and after adding 5 mL distilled water, the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions. Inhibition of enzyme activity was calculated as (%) = (A-C) ×100/ (B-C), where, A = absorbance of the sample, B = absorbance of blank (without α-amylase), and C = absorbance of control (without starch).

$$\% \text{ Inhibition} = \frac{A_{Sample} - A_{Control}}{A_{Blank} - A_{Control}} \times 100$$

Where, A_{Sample} = absorbance of test sample solution

$A_{Control}$ = absorbance of control solution

A_{Blank} = absorbance of blank solution

Determination of Total Phenolic Content of *N. lappaceum* (Kyet-mauk) Seeds by FCR Method

One of the antioxidative factors, total phenolic content (TPC) was measured by spectrophotometrically according to the Folin-Ciocalteu method (Reynertson, 2007). Frist, 1 mL of different concentration of Gallic acid solution (20, 10, 5, 2.5, 1.25 and 0.625 μg/mL) was mixed with 5 mL of diluted F-C reagent (FCR: H_2O, 1:10) and incubated for 5 min. To each tube, 4 mL of 1M sodium carbonate was added and the tubes were kept in room temperature for 15 min and the UV absorbance of reaction mixture was read at λ_{max} 765 nm. A standard curve

was prepared by plotting the absorbance against concentration of Gallic acid. The phenolic content in each sample was estimated by Folin–Ciocalteu method. Each extract (1 mg) was mixed with 1 mL of distilled water. To this, 5 mL of F-C reagent (1:10) was added and incubated for 5 min. To each tube, 4 mL of 1M sodium carbonate was added and the tubes were kept in room temperature for 15 min and the UV absorbance of reaction mixture was read at λ_{max}765 nm. The blank solution was prepared as the above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as µg Gallic acid equivalents (GAE)/mL of different extracts.

Determination of Antimicrobial Activity of Various Crude Extracts of *N.lappaceum* (Kyet-mauk) Seeds

Antimicrobial activities of various crude extracts such as PE, EtOAc, EtOH, and H_2O were investigated by agar well diffusion method at the Pharmaceutical Research Department (PRD), Ministry of Industry, Yangon.

The agar well diffusion method was used to test the antimicrobial action of the extracts on 24 h broth culture of the organisms used. Nutrient agar (4.6 g) and agar (1 g) were dissolved in 20 mL distilled water. The resulting nutrient agar medium was autoclaved at 121°C for 15 min and cooled in water bath (40 °C). After cooling, bacteria suspension of each bacteria strain (0.02 mL) was added and poured into Petri dishes. The seeded plates were allowed to dry in room temperature for 10 min. A standard cork borer of 10 mm diameter was used to cut uniform wells on the surface of the solid medium. The extracts of PE, EtOAc, EtOH, and H_2O (1 g) of each were dissolved in 1 mL of their respective solvent. Each of the different crude extracts (0.15 mL) were filled into the wells and incubated at 37 °C for 18-24 h. Antimicrobial activity in terms of zones of inhibition (mm) was recorded after 24 h of incubation (Lawrence *et al.*, 2009).

Determination of Cytotoxicity by Brine Shrimp Lethality Bioassay of *N.lappaceum* (Kyet-mauk) Seeds

Artificial sea water (9 mL) and (1 mL) of different concentrations of samples and standard solutions were added to each chamber of ice tray. Alive brine shrimp(10 nauplii) were taken with pasteur pipette and placed into each chamber. They were incubated at room temperature about 24 h. After 24 h, the number of dead or survive brine shrimp was counted and 50 % of lethality dose (LD_{50}) was calculated (Sahagal *et al*., 2010).

Results and Discussion

Phytochemical Constituents of *N. lappaceum* (Kyet-mauk) Seeds

According to the phytochemical tests in order to know their types present in the selected sample, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch, tannins, terpenoids and organic acids were found to be present. Cyanogenic glycosides and steroids were absent. Cyanogenic glycosides act as selective cytotoxic agents. Longer period of intake of the small number of cyanogenic glycosides in daily food have led to chronic toxicity.

Nutrients in *Nephelium lappaceum* L. (Kyet-mauk) Seeds

Nutrients in (Kyet-mauk) seeds sample were investigated by standard AOAC methods. The nutrients 7.30 % of moisture, 1.82 % of ash, 30.53 % of fat, 11.71 % of protein, 21.53 % of fiber, 27.11 % of carbohydrate and energy value 430.07 cal /100 g

were detected. So, rambutan seeds are not poisonous and contain carbohydrate, fats, proteins, which can meet the needs of the body of nutrients.

Elements Present in *N. lappaceum* (Kyet-mauk) Seeds by Energy Dispersive X ray Fluorescence (ED-XRF) Spectrometry

X-ray spectroscopy permits simultaneous analysis of light elements to heavy elements. Simadzu EDX-720 spectrometer can analyze the elements from Na to U under vacuum condition. In this work, relative abundance of elements present in seeds of *N.lappaceum* was determined by EDXRF spectrometer. K (49.528 %), Ca (38.818 %), Fe (4.651 %), Zn (2.264 %), Mn (1.892 %), Cu (1.752 %), and Rb (1.095 %) were observed. Among them, potassium content of the sample was the highest (49.528 %). High potassium decreases the risk of stroke, lower blood pressure, protects against loss of muscle mass, preserves bone mineral density and reduces the formation of kidney stones.

Antioxidant Activity of Ethanol and Watery Extracts of *N.lappaceum* (Kyet-mauk) Seeds by DPPH Radical Scavenging Assay

The antioxidant activity of ethanol and watery extracts of *N.lappaceum* seeds was evaluated by DPPH (1, 1-diphenyl-2-picryl-1-hydrazyl) radical scavenging assay. According to the procedure as described by (Marinova and Batchvarou, 2011), ascorbic acid was used as standard. Colorimetry with DPPH, a stable free radical, has been reported as a simple method for evaluation of the free radical scavenging activity. It tends to capture hydrogen from the antioxidant. Due to its free radical, the ethanolic DPPH solution is violet and absorbs at 517 nm. The colour changes upon neutralization of this free radical from violet to pale yellow by daylight. The decolouration of the initial colour is proportional to the test substances having anti-radicalizing power. Preliminary test for radical scavenging activity by DPPH method based on the change in colour of crude extracts. The absorbance of different concentrations (40, 20, 10, 5, 2.5, 1.25 μg/mL) of tested samples were measured at maximum absorption of wavelength 517 nm by using UV-7504 spectrometer. Absorbance was measured in triplicates for each solution. Ethanol extract (IC_{50} = 16.34 μg/mL) was found to be more potent than watery extract (IC_{50} = 17.31 μg/mL) comparing to standard ascorbic acid (IC_{50} = 2.25 μg/mL). The higher the antioxidant activity of ethanol extract of Kyet-mauk seeds possessed due to the lower IC_{50} value. Ethanol extract of Kyet-mauk seeds was found to be lower effective than standard ascorbic acid (IC_{50} = 2.25 μg/mL) (Table 1 and Figures 2 and 3).

Table 1 Percent Radical Scavenging Activity and IC_{50} Values of Ethanol and Watery Extracts of *N.lappaceum* (Kyet-mauk) Seeds and Standard Ascorbic Acid

Samples	% Inhibition (Mean ± SD) In Different Concentration (µg/mL)						IC_{50} (µg/mL)
	1.25	2.5	5	10	20	40	
Ethanol extract	7.550 ± 1.410	10.540 ± 0.403	26.070 ± 1.410	40.880 ± 1.007	55.270 ± 5.641	61.540 ± 0.403	16.34
Water extract	7.410 ± 0.403	9.690 ± 0.806	21.510 ± 0.202	39.740 ± 0.604	52.710 ± 0.806	59.120 ± 0.604	17.91
Ascorbic acid	34.360 ± 0.000	53.860 ± 0.000	62.380 ± 0.000	66.870 ± 0.000	72.750 ± 0.010	82.500 ± 0.000	2.25

IC_{50} = 50 % Inhibition Concentration

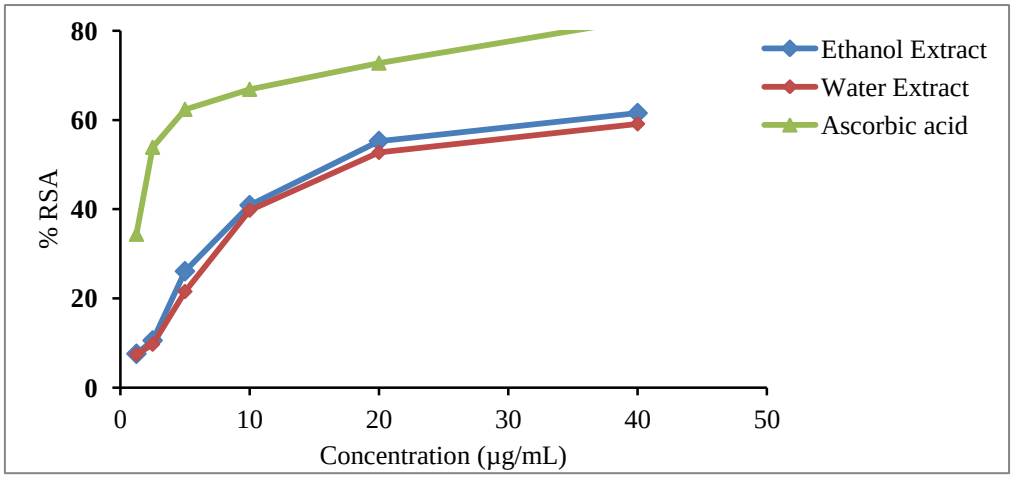

Figure 2 %RSA of ethanol and watery extracts of *N.lappaceum* (Kyet-mauk) seeds and standard ascorbic acid

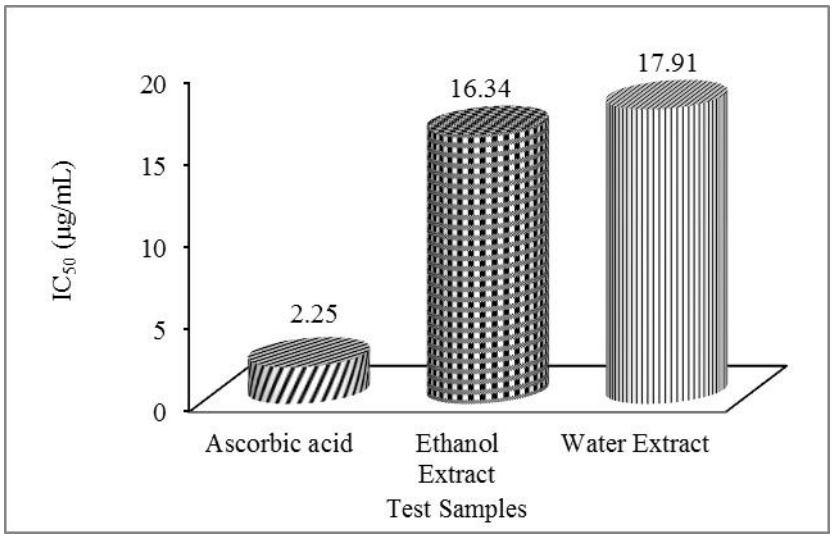

Figure 3 A bar graph of IC_{50} values of ethanol and watery extracts of *N.lappaceum* (Kyetmauk) seeds

α-Amylase Enzyme Inhibition Activity of Ethanol and Watery Extracts of *N. lappaaceum* (Kyet-mauk) Seeds

In the present study, α-amylase inhibitory activities of ethanol and watery extracts of *N.lappaaceum* (Kyet-mauk) seeds were investigated. The percentage inhibition of the α-amylase by ethanol and watery extracts were studied in a concentrations of 400, 200, 100, 50, 25, 12.25 µg/mL respectively. α-Amylase activity was measured *in vitro* by hydrolysis of starch in presence of α-amylase enzyme. This process was quantified by using iodine, which gives blue colour with starch. The reduced intensity of blue colour indicates the enzyme-induced hydrolysis of starch into monosaccharides. If the substance/extract possess α-amylase inhibitory activity, the intensity of blue colour will be more. In other words, the intensity of blue colour in test sample is directly proportional to α-amylase inhibitory activity. The treatment goal of diabetic patients is to maintain near normal levels of glycemic control, in both fasting and post-prandial conditions (Matsui *et al*., 2001). α-Amylase catalyzed the hydrolysis of α-1, 4-glycosidic linkage of starch, glycogen and various oligosaccharides. Alpha-glycosidase further breaks down the disaccharides to simple sugars, readily available for intestinal absorption. The inhibition of their activity in the digestive tract of humans is considered to be effective tool to control diabetes. In addition, these effects may lead to diminish absorption of monosaccharides (Hara and Honda *et al.*, 1990). Therefore, effective and nontoxic inhibitors of alpha-amylase and alpha-glucosidase have long been sought.

The percent inhibitions of α-amylase activity of ethanol and watery extracts were 80.58 µg/mL and 178.86 µg/mL that indicated the superiority of ethanol extract over watery extract (Table 2 and Figures 4 and 5). These extracts exhibited lower activity than standard acarbose (IC_{50} = 13.59 µg/mL). Inhibition of α-amylase could lead to reduction in postprandial hyperglycemia in diabetic condition (Mandal *et al*., 2016).

Table 2 α-Amylase Enzyme Inhibitions and IC_{50} Values of Ethanol and Watery Extracts of *N.lappaaceum* (Kyet-mauk) Seeds and Standard Acarbose

Sample	% Inhibition (mean ± SD) in different concentration (µg/mL)						IC_{50} (µg/mL)
	12.5	25	50	100	200	400	
Water extract	17.647 ± 0.003	21.307 ± 0.008	34.756 ± 0.006	39.809 ± 0.006	52.727 ± 0.026	56.604 ± 0.025	178.86
Ethanol extract	21.929 ± 0.004	35.315 ± 0.008	41.667 ± 0.009	55.285 ± 0.008	65.000 ± 0.012	71.569 ± 0.001	80.58
Std-Acarbose	48.110 ± 2.270	69.800 ± 1.900	73.150 ± 0.630	83.000 ± 4.110	90.160 ± 4.430	93.060 ± 2.210	13.59

IC_{50} = 50 % Inhibition Concentration

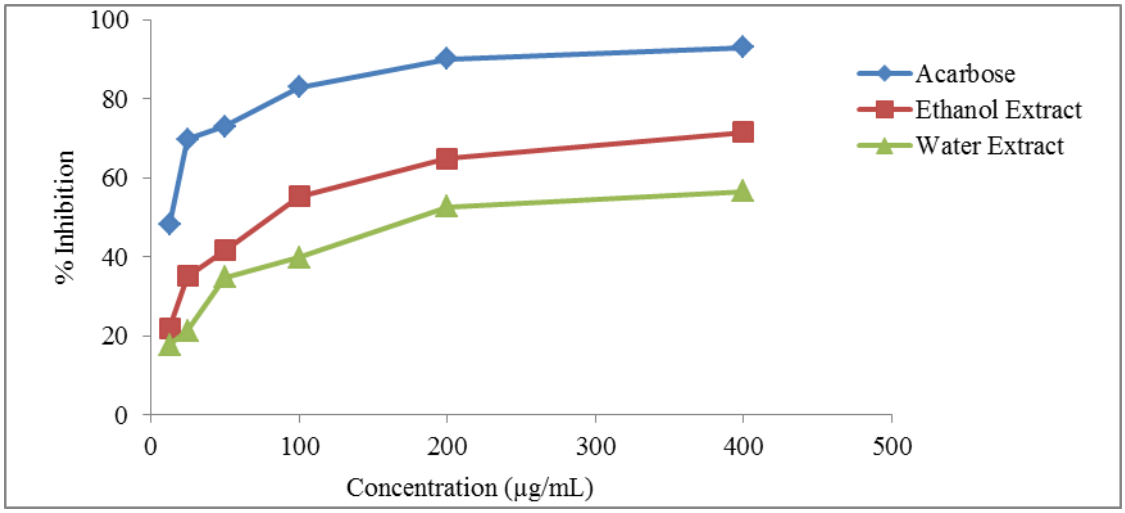

Figure 4 α-Amylase enzyme inhibition activities of watery and ethanol extracts of *N.lappaaceum* (Kyet-mauk) seeds and standard acarbose

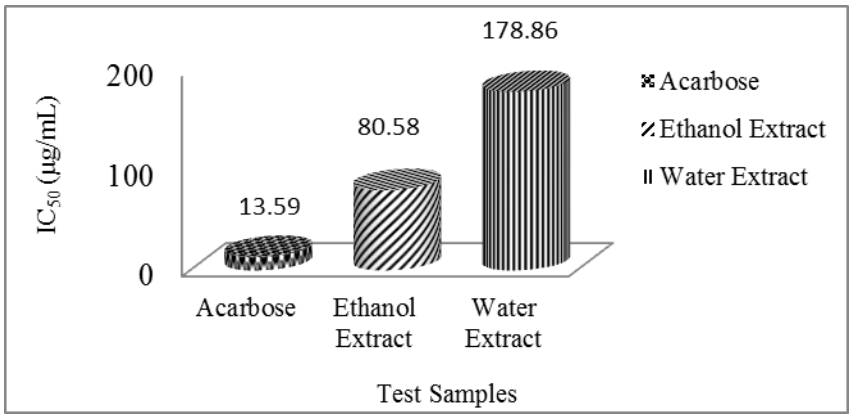

Figure 5 A bar graph of IC_{50} (µg/mL) of watery and ethanol extracts of *N.lappaaceum* (Kyet-mauk) seeds and standard acarbose

Total Phenol Contents of Ethanol and Watery Extracts of *N. lappaceum* (Kyet-mauk) Seeds by Folin-Ciocalteu Reagent (FCR)

In this study, the total phenolic contents of (Kyet-mauk) seeds were measured at 765 nm colorimetrically by Folin-Ciocalteu method. The TPC content of ethanol extract (79.88 ± 2.86 µg GAE/mg) was found to be higher than watery extract (30.73 ± 7.65 µg GAE/mg). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. They possessed biological properties such as antigaging, anticarcinogen and antiflammation (Zainol *et al*., 2003). These correlations indicated that high total phenol contents in ethanol extract contributed to high in antiradical scavenging activity and α-amylase inhibition activity of ethanol extract (Table 3 and Figure 6).

Table 3 Total Phenol Content (TPC) of Ethanol and Water Extracts of *N. lappaaceum* (Kyet-mauk) Seeds

No.	Extracts	TPC (µg GAE/mg ± SD)
1	Ethanol extract	79.880 ± 2.860
2	Water extract	30.730 ± 7.650

GAE = Gallic acid equivalent

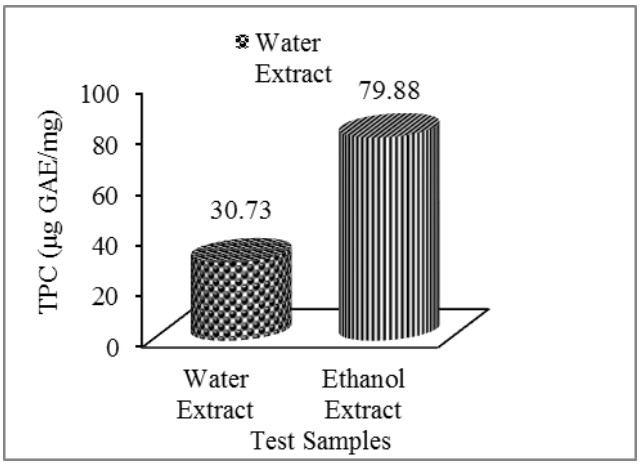

Figure 6 A bar graph of total phenolic contents of ethanol and watery extracts of *N. lappaaceum* (Kyet-mauk) seeds

Antimicrobial Activity of Various Crude Extracts of *N. lappaceum* (Kyet-mauk) Seeds by Agar Well Diffusion Method

Antimicrobial activities of *N. lappaceum* (Kyet-mauk) seeds crude extracts such as PE, EtOAc, EtOH and H_2O were investigated by agar well diffusion method. The microorganisms tested were *B. subtilis, S. aureus, P. aeruginosa, B. pumilus, C. albicans and E. coli.* The diameter of agar well was 10 mm. When comparing different antimicrobial agents to known concentration, the inhibitory zone diameter is taken as a measure of antimicrobial activity. The larger the diameter, the higher the antimicrobial activity of the test agents. EtOH extract showed the antimicrobial activity with the inhibition zone diameter range between 10~14 mm against six microorganisms such as *B. subtilis, S. aureus, P. aeruginosa, B. pumilus, C. albicans and E. coli*. EtOAc extract inhibited all microorganisms. Water extract inhibited five microorganisms except *E. coli*. PE extract showed the activity on *B. subtilis*, *S. aureus*, *P. aeruginosa, C. albicans* and non-activity on other microorganisms. Ethanol extract had more antimicrobial activity than EtOAc, watery and PE extracts (Table 4).

Table 4 Inhibition Zone Diameter of Various Crude Extracts of *N. lappaceum* (Kyet-mauk) Seeds

No.	Microorganisms	Diameter of inhibition zone (mm)			
		PE extract	EtOAc extract	EtOH extract	H_2O extract
1.	*Bacillus subtilis*	11 (+)	11 (+)	11 (+)	11 (+)
2.	*Staphylococcus aureus*	11 (+)	11 (+)	12 (+)	12 (+)
3.	*Pseudomonas aeruginosa*	11 (+)	12 (+)	13 (+)	14 (+)
4.	*Bacillus pumilus*	−	11 (+)	11 (+)	12 (+)
5.	*Candida albicans*	11 (+)	11 (+)	12 (+)	11 (+)
6.	*Escherichia coli*	−	11 (+)	12 (+)	−

Diameter of agar well = 10 mm 10 mm ~ 14 mm (+)

Cytotoxicity of Ethanol and Watery Extracts of *N. lappaceum* (Kyet-mauk) Seeds by Brine Shrimp Lethality Bioassay

The brine shrimp assay is very useful tool for the isolation of bioactive compounds from plant extracts (Ramachandran, 2011). The method is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test in the micro well scale. It is considered as useful tool for preliminary assessment of toxicity. It has also been suggested for screening pharmacological activities of plant extracts (Prashith, 2012).

The ten nauplii of brine shrimp (*Artemiasalina*) are used for each chamber. The cytotoxicity effect was expressed as LD_{50} values (50% Lethality Doses). The percentage of dead brine shrimp in 1000, 100, 10, 1 µg/mL concentration for watery extract were 36.67 %, 26.67 %, 16.67 %, 6.67 % and ethanol extract were 46.67 %, 30.00 %, 23.33%, 13.33%. LD_{50} values of both watery and ethanol extracts were $LD_{50} > 1000$ µg/mL(Table 5). Standard Caffeine did not show cytotoxicity until 1000 µg/mL concentration whereas cytotoxicity of standard $K_2Cr_2O_7$ was LD_{50} = 55.01 µg/mL. According to results, the rambutan seeds are not poisonous until 1000 µg/mL concentration. The LD_{50} was reported to be greater than 5000 mg kg^{-1} of extract (Rohman, 2017).

Table 5 Cytotoxicity of Different Concentration of Ethanol and Watery Extracts of *N. lappaceum* (Kyet-mauk) Seeds

Test samples	% of Dead brine shrimp in different concentration of sample				LD_{50} (µg/mL)
	1	10	100	1000	
Ethanolextract	13.33	23.33	30.00	46.67	>1000
Wateryextract	6.67	16.67	26.67	36.67	>1000
$K_2Cr_2O_7$	40.33	43.33	56.67	100.00	55.01
Caffeine	0	0	0	0	0

No. of brine shrimp = 10

Conclusion

The research finding revealed the presence of alkaloids, α–amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugar, saponin, starch, tannin and terpenoids. Cyanogenic glycosides and steroids were absent in Kyet-mauk seeds. The nutritional values determined by AOAC method indicated that 7.30 % of moisture, 1.82 % of ash, 30.53 % of fat, 11.71 % of protein, 21.53 % of fiber and 27.11 % of carbohydrate based on the dry weight. And also 430.07 kcal/100g of energy value was observed in Kyet-mauk seeds. The mineral contents such as K, Ca, Fe, Zn, Mn, Cu and Rb of Kyet-mauk seeds powder sample were quantitatively determined by EDXRF. Among them, the potassium content of this sample was the highest (49.528 %).

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay showed that ethanol extract (IC_{50} = 16.34 µg/mL) of Kyet-mauk seeds was more effective than watery extract (IC_{50} = 17.91 µg/mL) in antioxidant activity and it was also observed to be comparable with the standard ascorbic acid (IC_{50} = 2.25 µg/mL). The percent inhibition of *in vitro* α-amylase inhibition activity of ethanol and watery extracts of (Kyet-mauk) seeds was investigated by starch-iodine method. The IC_{50} values of ethanol and watery extracts were found to be 80.58 µg/mL and 178.86 µg/mL respectively. These activities of extracts were found to be lower than that of standard acarbose (IC_{50} = 13.59 µg/mL). The total phenol content (TPC) of ethanol and watery extracts of Kyet-mauk seeds were determined by FCR method. According to the results, the superiority of antioxidant and α-amylase inhibition activities of ethanol extracts over watery extracts of Kyet-mauk seeds was correlated with the total phenol content of ethanol extract.

EtOH and EtOAc extracts showed the antimicrobial activity with the inhibition zone diameter range between 10 ~ 14 mm against all six microorganisms - *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, B. pumilus, C. albicans and E. coli*. Water extract inhibited five microorganisms except *E. coli.* PE extract showed the activity on *B. subtilis, S. aureus, P. aeruginosa, C. albicans* and non-activity on other microorganisms.

According to cytotoxicity test it was observed that the LD_{50} values of both ethanol and watery extracts were LD_{50}>1000 µg/mL. Therefore, the Kyet-mauk seeds fat and oil extracts are practically non-toxic.

Since previous and present studies suggest that *N. lappaceum* L. (Kyetmauk) seeds have shown potential as sources of natural antioxidants, further studies need to be directed to isolate and characterize antioxidant active compounds from the extracts which could be responsible for antioxidant and antidiabetic activities. Finally, the present study provided to justify the traditional claim of herbs for antioxidant, antimicrobial and antidiabetic activities. This plant may essentially contain herbal bioactive compounds, inhibitory enzyme activities and some bioactivities.

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