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Antioxidant and hypoglycemic activities of extract and fractions of Rambutan seeds (*Nephelium lappaceum* L.)

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ABSTRACT

Objective : This research was done to measure the antioxidants and hypoglycemic activities of NLS extract and fractions.

Methods: The ethanol extract of rambutan seeds were prepared by maseration method and the fractions (n-hexane, ethyl acetate, buthanol and water) by separation of extract based on the polarity. The antioxidant activity was determined by using superoxide dismutase value (SOD), 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity. The hypoglycemic activity was determined by using inhibition of α-glucosidase test. The DPPH scavenging and hypoglycemic activities were analized by median of Inhibitory Concentration (IC-50).

Result : The highest SOD activity showed that ethyl acetate and aqueous fraction of NLS were 3.3771 µg/ml and 3.0374 µg/ml. Meanwhile DPPH assay showed that both NLS extract and fractions had low DPPH scavenging activity. Hypoglycemic activity showed that extract of NLS had highest activity as α-glucosidase inhibitor.

Conclusion : NLS extract and fractions have high SOD antioxidant value but low DPPH scavenging activity and can be used as potential hypoglycemic agent.

1. Introduction

Diabetes Mellitus (DM) is common disease associated with markedly increase mortality rate. The number of DM has reached to 285 million, approximately 6.4% of the world's adult population, more than 3.8 million people die yearly [1,2,3]. Diabetes can be characterized by hyperglycemia resulting from defects in insulin action and insulin secretion or both. The most common type of DM is DM II, which accounts for 85 to 95% of all cases and constitutes a major and growing public health problem [4]. DM II is generally managed through intensive therapy that consists of lifestyle and sequential addition of oral antihyperglycemic agents (OHAs). One therapeutic approach to decrease postprandial hyperglycemia in diabetic state is by retarding absorption of glucose through inhibition of carbohydrate hydrolyzing enzymes, like α-glucosidase and α-amylase in the digestive tract [5]. α-glucosidase inhibitors delay absorption of complex carbohydrates and thus inhibit postprandial glucose peaks thereby leading to decreased postprandial insulin levels [6].

Oxidative stress in hyperglycemia-related diabetic patient causing excessive amount of free radical which may damage antioxidant defense [7].

These drugs can induce hypoglycemia, lose their efficacy, have prominent side effects and trigger diabetic complications. Plants have been suggested as source of potentially antidiabetic drugs [8].

Nephelium lappaceum L. known as rambutan is one of variety tropical fruit which commonly consumed in south-east Asia. Rambutan has antioxidant activity and high phenolic content [9].

Therefore, we conducted our research to evaluate antioxidant and hypoglycemic activty of rambutan's seed (NLS) extract and fractions.

2. Materials and Methods

2.1 Extract and fraction preparation

Extraction was done based on maceration method [10,11,12] and fractions were done as modified partition [10,13]. NLS was collected from Kesamben-Blitar plantation, East Java, Indonesia. Four hundred gram of dried and milled NLS were soaked in destilated ethanol 70% then were evaporated and resulted 11.25 % of crude extract (45 g). Fifty gram of NLS ethanolic extract was partitioned with n-hexan and water (1:1), yielded hexane fraction 23.37 g (46,74 %), The residue was partitioned with ethyl acetate and water (1:1) yielded ethyl acetate fraction 2.3 g (7.71%), the residue was partioned with buthanol and water (1:1) yielded buthanol fraction 2.85 (9.56 %), the residue was water fraction 1.3 g (2.6 %).

2.2 DPPH scavenger test

The DPPH scavenger test was done by introducing 50 µl extract and fractions of NLS in methanol with final concentrations (0.19 µg/ml; 0.391; 0.781; 1.563; 3.125; 6.25; 12.5; 25; 50; to 100 µg/ml) in 96 well microplate and were added 200 µl DPPH 0.077 mmol in DMSO. The mixture was shaken vigorously and incubated at room temperature and dark room for 30 min, and then measured at 517 nm absorbance using a microplate reader (Multi Go Skan). Negative controls used DPPH 250 µl, blank used 250 µl methanol [11,12,13] . The DPPH scavenger activity (%):

$$\text{scavenging } \% = \frac{A_c - A_s}{A_c} \times 100$$

Aa: sample absorbance
Ac: negative control absorbance (without sample)

2.3 Superoxide Dismutase (SOD) test

The SOD test was done by a SOD assay kit (Cayman) comprised of assay buffer, sample buffer, radical detector, SOD standard, and xanthine oxidase. SOD standards were prepared by introducing 200 µl diluted radical detector and 10 µl SOD standard (7-level standard) per well [12]. Samples were dissolved in DMSO in concentrations 500, 125, 31.25 µg/ml. The sample well contained 200 µl diluted radical detector and 10 µl sample. Samples and standards wells were added 20 µl diluted xanthine oxidase. The mixtures were shaken carefully for few seconds, incubated for 20 minutes at room temperature, SOD activity was measured on a microplate reader at 440-460 nm [12]. The SOD value was calculated using the equation from the linear regression of standard curve substituting linear lineared rate (LR) for each sample. One unit is defined as the amount of enzyme to yield 50% dismutation of the superoxide radical [12]. Furthermore, standard curves were constructed based on the value of the LR and SOD value be calculated:

The calculation was done by calculating the value of SOD linearized rate/LR (LR Std A= Abs Std A/Abs Std A: LR Std B= Abs Std A/Abs Std B)

2.3 α-glucosidase inhibitor test

The α-glucosidase inhibitor activity was

$$\text{SOD (U/ml)} = \left[\left(\frac{\text{Sample LR-y intercept}}{\text{Slope}}\right) \text{X } 0{,}23\text{ml}/0{,}01\text{ml}\right] \text{X Sample dilution}$$

tested by the modified method of [14,15]. Samples were diluted in DMSO 10% with various concentrations (500 µg/ml; 125; 31.25; 7.81; 1.9 µg/ml). Amount of 5 µL of sample, 25 µl of 200 mM p-nitrophenyl- a-glucopyranoside, 45 µl phosphate buffer saline (pH 7), 25 µl of *Saccharomyces* sp. yeast α- glucosidase were introduced in the microplate and incubated at 37^0 C for 5 min. The reaction were stopped by adding 100 µL of 200 mM Na_2CO_3 and then was measured at 400 nm using a microplate reader (Multi Go Skan). Controls without inhibitors were checked, as a reference. The α-glucosidase inhibitory activity could be calculated as follows:

$$\text{scavenging } \% = \frac{A_c - A_s}{A_c} \times 100$$

Ac, as represent the absorbance at 400 nm of the control, sample respectively

3. Result

3.1 DPPH scavenger activity

The DPPH free radical scavenger activity of extract and fractions are representative of antioxidant activity. The IC_{50} is the concentration of antioxidant needed to scavenge 50% of the DPPH free radical [12]. Based on the IC_{50} (Table

1.) and Figure 1 showed that NLS extract had the lowest DPPH scavenger activity with IC_{50} 341.20 µg/ml, ethyl acetate fraction had highest activity with IC_{50} 104.03 µg/ml.

Based on the IC_{50} values of DPPH scavenger activity (Table 1.) showed that extract and fractions of NLS had low antioxidant activity. The IC_{50} value was used to determine an antioxidant

Table 1. DPPH scavenger activity (IC_{50}) of extract and fractions of NLS. The DPPH scavenger activity test were measured triplicate for each sample. Linear equations, coefficient of regression (R^2), and IC_{50} were calculated.

Samples	Linear equation	R^2	IC50 (µM)
Extract	Y=0.1415X+1.7203	0.9285	341.20
Hexane fraction	Y=0.2842X+4.3993	0.9002	160.45
Ethyl acetate fraction	Y=0.4519X+2.9909	0.9887	104.03
Buthanol fraction	Y=0.155X+3.9125	0.8904	297.34
Water fraction	Y=0.2188X+5.6823	0.9023	202.55

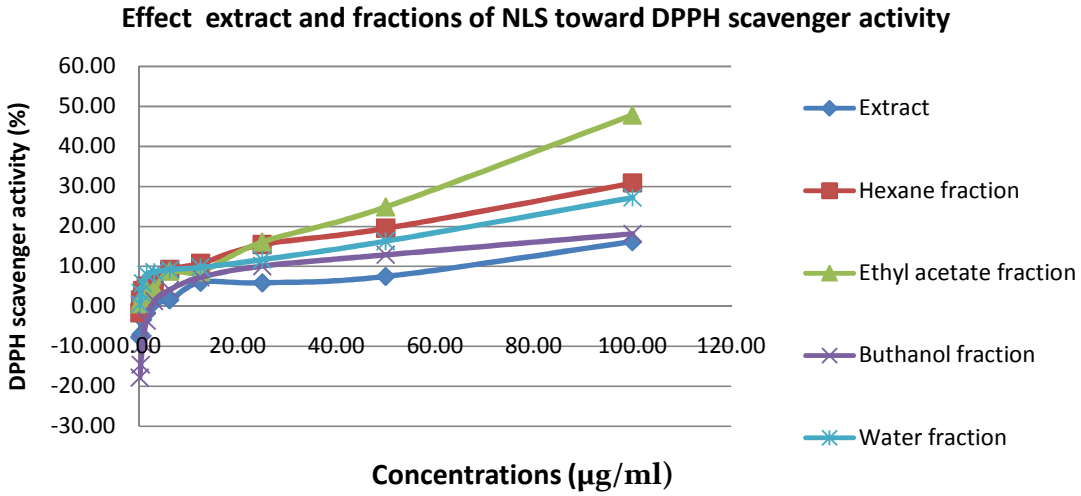

Figure 1. DPPH scavenger activity of extract and fractions of NLS diluted in methanol to achieve the final concentrations 100; 50; 25; 12.5; 6.25; 3.125; 1.563; 0.781; 0.391; 0.19 µg/ml.

activity by DPPH test, which the smallest the IC_{50} value is highest antioxidant activity [13].

3.2 The SOD activity

Superoxide anion (O_2^{*-}) is one of the most important radical formed in aerobic cells due to leakage of the electron transport chain. Although less reactive but radical O_2^{*-} is initiation radical oxidation, it is a precursor to form hydroxyl radical (OH^*) are highly reactive through Fenton reaction and Haber-Weiss [16]. Superoxide dismutase (SOD) as an antioxidant activity would be increased by changing the superoxide anion (O_2^{*-}) into hydrogen peroxide (H_2O_2) and oxygen (O_2) [17]. The SOD activity of extract and fractions of NLS in trapping O_2^{*-} can be seen in Table 2, Figure 2.

Based on the results (Table 2.) showed that lower concentrations of sample reduced SOD value. The highest SOD value at a concentration of 500 µg/ml was buthanol fraction (3.377 U/ml), while the lowest was hexane and ethyl acetate fractions (1.334-1.472 U/ml). The highest SOD

Table 2. Mean and Tukey HSD post hoc test of SOD activity of extract and fractions (U/ml) was measured in triplicate for sample. (Linear equation, coefficient of regression (R^2) of SOD standard and SOD activity of extract and fractions were calculated)

Samples	Concentrations (µg/ml)		
	500	125	31.25
Extract	1.780±0.118 b	1.296±0.036 c	0.807±0.032 bc
Hexane fraction	1.334±0.091 a	1.038±0.014 b	0.726±0.033 b
Ethyl acetate	1.472±0.164 a	0.759±0.005 a	0.577±0.052 a
Buthanol fraction	3.377±0.175 d	1.874±0.077 d	0.876±0.026 c
Water fraction	3.037±0.095 c	2.314±0.024 e	1.587±0.049 d

Data are presented as mean ± standard deviation. Different letters in the same column (among samples)are significant at $P < 0.05$ (Tukey's HSD post hoc test)

value at 125 µg/ml was water fraction 2.314 U/ml and the lowest was ethyl acetate fraction 0.759 U/ml. The highest SOD value at 31.25 µg/ml was water fraction 1.587 U/ml), the lowest was ethyl acetate (0.577 U/ml). Overall in the three concentrations the highest SOD value were buthanol and water fractions of NLS. Table 2 showed that the higher concentration was used the higher sample could reduce SOD value.

3.3 The α-glucosidase inhibitor activity

Alpha-glucosidase is a key enzyme in carbohydrate digestion, it catalyzes the hydrolysis of 1,4-α-glucosidic bonds within carbohydrates with release α-glucose and trigger the gaining blood glucose levels after meal. Alpha-glucosidase inhibitors can delay the intestinal carbohydrate

Table 3. α-glucosidase inhibitor (IC_{50}) of extract and fractions of NLS. The α-glucosidase inhibitor activity test were measured triplicate for each sample. Linear equations, coefficient of regression (R^2), and IC_{50} were calculated.

Samples	Linear equation	R^2	IC50 (µM)
Extract	Y=1.8448X+31.705	0.9507	9.92
Hexane fraction	Y=2.6678X+6.7865	0.8728	16.20
Ethyl acetate fraction	Y=2.2174X+26.866	0.8003	10.43
Buthanol fraction	Y=2.7092X+15.662	0.8587	12.67
Water fraction	Y=2.5254X+14.2	0.8324	14.18

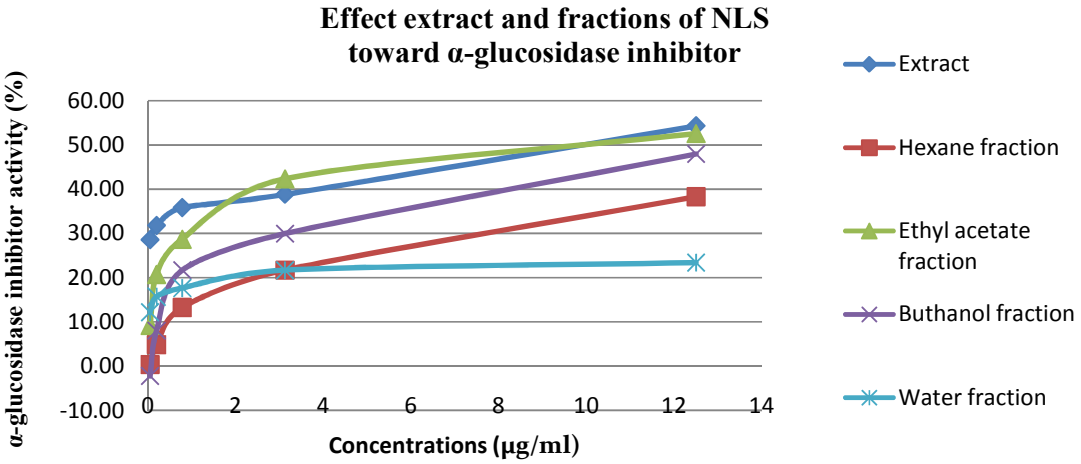

Figure 2. α-gucosidase inhibitor activity of extract and fractions of NLS diluted in DMSO to achieve the final concentrations 12.5; 3.125; 0.781; 0.195; 0.049 µg/ml

absorption and slow the gaining blood glucose levels [3]. The α-glucosidase inhibitors of extract and fractions can be seen in Table 3, Figure 2.

Based on (Table 3) showed that extract and fractions of NLS had high α-glucosidase inhibitor activity. The highest α-glucosidase inhibitor activity was extract with IC_{50} 9.92 µg/ml.

4. Discussion

Extract and fractions of NLS had low DPPH free radical scavenger activity (Table 1., Fig. 1), but had high SOD value (Table. 2). Based on Table 1 showed that NLS seed extract had lowest radical scavenging activity. This data was consistent with previous research that rambutan seed contained low of total phenolic compound, rambutan peel extract contained 542.2 mg catechin/g and rambutan seed extract contained 58.5 mg catechin/g. The rambutan peel extract exhibited higher antioxidant activity than seed extracts [18], rambutan peel possessed high DPPH free radical scavenging activity (IC_{50} of 8.87 µg/ml) [19], high polyphenols content of rambutan peel contributes towards high free radical scavenging activity [20]. The result of DPPH assay in our study showed that ethyl acetate fraction which had the highest free radical scavenging activity with IC_{50} 104.3 µg/ml, in previous study the highest DPPH scavenger activity was methanolic fraction with IC_{50} 4.94 µg/ml [21]. While the reason for the different activity could be due to different solvent for extraction and fractionation [22]. Previous study

exhibited that ethanolic extract of white saffron had lower DPPH scavenger activity compared to water extract of white saffron [22, 23]. Different solvent resulted different compound and bioactivity. Water extract of Forsythia korean flowers exhibited a higher phenolic content than ethanolic extract [24].

Previous studies reported that rambutan seed possessing a relatively high amount of fat with values between 14-41 g/100 g [25], the rambutan seed possesses a relatively high amount of fat between 17-39%. The fat content of rambutan seed were saturated fatty acid (SFA) 50.7% and monosaturated fatty acid (MUFA) 48.1%, the main fatty acid were oleic acid 40.3%, arachidic acid 34.5%, stearic acid 7.1% [26]. Previous data that using HPLC of rambutan seed contained high triacyglycerol with AOO (Arachidoyl-Dioleoylglycerol) 49.84%, ASO (Arachidoyl-Stearoyl-Oleoylglycerol) 15.058%, AOP (Arachidoyl-Oleoyl-Palmitoglycerol) 12.822% [27]. The natural antioxidant in lipid-containing product and lipid-based product such as oil, fat, margarine, butter in rambutan seed are considered insufficient antioxidant activities [26], fermentation and roasting process in rambutan seed fat can improve the antioxidant activity and total phenolics compound of rambutan seed fat [26,28].

Extract of NLS showed the highest α-glucosidase inhibitor activity (Table 3., Figure 2.). This data was validated with previous research that high dose of rambutan seed infusion (3.12 g/kg bw) had significant effect in reducing the blood glucose and improve pancreatic beta cells of diabetic mice [29]. Extract and fractions of NLS were more active as α-glucosidase inhibitor activity compared to drug acarbose with IC_{50}=3500 µg/ml [30], more active than glucobay with IC_{50} 24.44 µg/ml [15]. α-glucosidase is an enzyme for carbohydrate digestion and absorption and has been used as therapeutic target for its modulating action to reduce postprandial hyperglycemia. Hyperglycemia is a risk factor for the development of oxidative stress-related diabetes mellitus [28, 29]. In this present study showed that NLS extract and fractions have potency to be hypoglycemic agent due to it α-glucosidase inhibitor activity.

5. Conclusion

This study has demonstrated the antioxidant and α-glucosidase inhibitory activities of NLS extract and fractions. The potential antioxidant activities are ethyl acetate fraction for highest DPPH radical scavenger activity and water fraction, buthanol fraction for highest SOD value. NLS extract and fractions have benefical effect to be hypoglycemic agent by inhibiting α-glucosidase activities.

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Conflict of Interest

The authors report no conflicts of interest

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