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Original Article

Antioxidant and Antidiabetes Capacity of Hexane, Ethylacetate and Ethanol Extracts of *Durio zibethinus* Murr. Root

Yayu Mulsiani Evary[1*], Amir Muhammad Nur[2]

ABSTRACT

Introduction: Active natural products from medicinal plants are good sources for the antioxidant and antidiabetic agent. Natural antioxidants protect human health against oxidative stress and free radicals which cause degenerative disease such as diabetes. **Objectives:** This study was conduct to try one approach to treat diabetes that is to reduce gastrointestinal glucose absorption through the inhibition of the α-glucosidase enzyme. **Methods:** We make three different root extracts from *Durio zibethinus* Murr. The extracts were examined for their DPPH radical scavenging activity, antioxidant activity using β-carotene-linoleic acid assay, and their α-glucosidase inhibitory activity by a standard method. Additionally, we have also determined their total polyphenols and total flavonoids quantitatively using spectrophotometer UV-Vis. **Results:** The root extracts *Durio zibethinus* Murr. were effectively scavenged DPPH radicals in varied rate. The hexane (EHD), ethyl acetate (EEAD), and ethanol (EED) extracts revealed DPPH radical scavenging activity with IC_{50} of 541.28, 83.95 and 11.24 µg/ml respectively and their β-carotene-linoleic acid assay showed activity with IC_{50} of 273.58, 139.53, and 166.83 µg/ml, respectively. *In vitro* assay of the α-glucosidase inhibitory activity of the EHD, EEAD, and EED extracts showed an IC_{50} of 119.84, 23.69, and 3.35 µg/ml, respectively.
Conclution: In this present study, we found that ethanol extract revealed the most active antioxidant activity and the highest inhibitory activity against α-glucosidase enzyme. The total phenolics and total flavonoids contents of the extracts were studied, where the ethanol extracts were found to have the highest than that of other extracts. This study proofs the medicinal potencies of *Durio zibethinus* Murr. root extracts.
Key words: *Durio zibethinus* Murr., antioxidant, α-glucosidase inhibitor, extracts, roots.

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INTRODUCTION

The normal metabolic process resulted the formation of Reactive Oxygen Species (ROS) and free radicals in human body.[1] The high formation of ROS could lead to human disease including diabetes and its complications.[2] The high incidence of diabetes make it be an urgent health problem to cure. The most frequently diabetes incidence is type II diabetes which showed with the high postprandial glucose concentration. One of possible mechanisms of medicinal plants to cure diabetes is reducing postprandial glucose concentration by inhibiting the α-glucosidase enzyme in the intestinal surface.[3]

Bioactive compounds from plants have searched for their antioxidant and antidiabetic properties. The large quantity of Polyphenols plants exhibited high antioxidant and α-glucosidase activity.[4] Some antioxidant mechanism has been found to have a good relationship with the α glucosidase inhibitory activity of medicinal plants, especially for DPPH scavenging activity and inhibiting lipid peroxidation. A research has been proof that the high DPPH and hydroxyl radical scavenging activity of *Murraya koenigii* leaf extracts resulted the high of their α-glucosidase inhibitory properties.[5]

Some medicinal properties of *Durio zibethinus* Murr. have been found early. The chloroform extract of *Durio zibethinus* fruit pulp and wood bark had been found to show a great antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.[6,7] The other medicinal properties of *Durio zibethinus* Murr. including antioxidant and anti-inflammatory effect of the fruit pulp,[8] antidiabetic and antihipercholesterolemia activity of its fruit peel extract.[9]

In this study, we determine the DPPH scavenging activity and inhibition of β-carotene bleaching activity of difference *Durio zibethinus* Murr. root extracts. In addition, we examine the α-glucosidase inhibitory activity of the extracts, *in vitro*. We also perform phytochemical identification of the extracts.

MATERIALS AND METHODS

Chemicals

1, 1 diphenyl-2picril-hydrazyl (DPPH), α-glycosidase from *Saccharomyces cerevisiae* and para-nitrophenyl-glucopyranoside were products of Sigma-Aldrich.

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Other chemicals and reagents used were of analytical grade while the water used was glass-distilled.

Plant material

Durio zibethinus Murr. root was obtained from South Sulawesi, Indonesia.

Preparation of extract

The fresh *Durio zibethinus* Murr. root was washed, dried (using herbs drier) and ground using wood machine. The dried sample was extracted using terraced extraction method with hexane, ethylacetate and ethanol sequentially, by maceration on a sonicator at room temperature and solvent was replenished every 24 h for 3 days to ensure that all possible compounds were extracted. The resulted extracts were filtered and concentrated using a rotary evaporator (Buchi) under reduced pressure at approximately 50°C. The concentrated extracts were air dried using electric fan until they dried well.

Phytochemical constituens determination

Total Phenolic Content

Total phenolic content of the extracs were examined by Folin-Ciocalteu method with slight modification.[10] The extract were prepared in methanol and 100 µl of each concentration in 5 ml volumetric flask was added with 2.5 ml Folin Ciocallteu's reagent (7,5%). The mixtures were vortexted and incubated at room temperature for 10 min. After incubation, 2 ml of NaOH 1% was added and the mixture was vortexted. The volume was adjusted to 5 ml and incubated for 1 h in the dark room. The absorbance was measured at 750 nm against blank with a UV/Vis spectrofotometer (UV 1800 Shimadzu, Japan). Gallic acid was used as standard. The results were expressed as mg gallic acid equivalents (GAE)/g extract. The determination of total penolic compound in the extracts were done in triplicate.

Total Flavonoid Content

Total flavonoid content was determined using aluminium-chloride colorimetric assay.[11] Briefly, 1 ml of extract solution in suitable solvent was mixed with 1.5 ml ethanol, 100 l of $AlCl_3$ (10%) and 100 µl of sodium acetate 1M, respectively. The volume was adjusted to 5 ml with ethanol in a volumetric flask and incubated for 30 min. Quercetin was used as standard. The absorbance was measured at 431 nm with a 1800-UV/Vis spectrofotometer (Shimadzu). All measurements were done in triplicate. Total flavonoid content was expressed as mg of quercetine equivalent (mgQE) per gram extract.

DPPH Radical Scavenging Assay[12]

Evaluation of antioxidant activity was done using DPPH radical scavenging method. In brief, 10mg/ml solution of DPPH in methanol was prepared. This solution (100 µl) was added to 20 µl of extracts with concentration of 10, 50, 100, 150, 200 ppm. Solutions were made up to 200 µl with methanol in a 96 well plate. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min, in a dark room. The absorbance was measured at 515 nm by using Biotek micro plate reader. Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using following equation:

$$\text{DPPH scavenging effect (\%) or Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 was the absorbance of control reaction and A_1 was the Absorbance in presence of test or standard sample.

β-Carotene-Linoleic acid Assay

β-Carotene-Linoleic acid Assay was done according to the method developed by Mammadov[13] with slight modification. Three mililiter of β-carotene solution (0.2 mg/ml in chloroform) was pippeted into a flask (100 ml) containing 0.12 ml linoleic acid and 1.2 ml of 100% Tween 20. The mixture was evaporated 40°C with agitation for 10 min to remove chloroform. After evaporation, the mixture was immediately diluted with 50 ml distilled water. The distilled water was added slowly to the mixture with vigorous agitation to form emulsion. The assay was done in 96 microplate. Into 180 µl of β-carotene emulsion was added 20 µl of extract solution in 5 different concentration. The mixture was gently shaked using thermomixer at 50°C for 120 min. Absorbance was measured at 450 nm using Biotek Microplate Reader. Blank solution was prepared containing β-carotene emulsion without sample. The total antioxidant activity was calculated using the following equation :

$$AA = (1 - (A_b^0 - A_b^t)/ (A_s^0 - A_s^t)) \times 100\%$$

Where AA is antioxidant activity, A_b^0 and A_s^0 are the absorbance of blank and sample at the initial time of incubation respectively. While A_b^t and A_s^t are absorbance of blank and sample at 120 min of incubation.

α-Glycosidase inhibitory assay [14]

The α-glucosidase inhibitory activity was determined by measuring the release of 4-nitrophenol from P-nitrophenyl α-D-glucopyranoside. The assay mixtures for these experiments contained 20 µl of sample (extracts) in different concentration added with 14 µl of 5 mM p-nitrophenyl α-glucopyranoside and 52 µl of phosphate buffer pH 7. The mixtures were pre-incubated for 5 min at 37°C. After that 14µl of enzyme solution 0.5 U/ml was added and incubated for 15 min at 37°C. The reaction was terminated by the addition of 100 µl of 100 m Msodium carbonate. The liberated p-nitrophenol was determined at 405 nm using Biotek Microplate Reader. The % inhibition rates were calculated using the formula,

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{405}(\text{control}) - \text{Abs}_{405}(\text{extract}) \times 100}{\text{Abs}_{405}(\text{control})}$$

The IC_{50} values were determined from plots of percent in hibition versus log inhibitor concentration. All tests were performed in triplicate.

Statistical Analysis

All data were analyzed by analysis of variance (ANOVA)

RESULTS AND DISCUSSION

The bioactive compound is summarized in Table 1, antioxidant activity assay in Table 2 and the *in vitro* α-glucosidase inhibitory activity in Table 3. The quantitative chemical compound analysis of *Durio zibethinus* showed that the root is rich in total phenolics and total flavonoids content according to the data shown in Table 1. Total Phenolics content of different extracts was significant and varying between 19.55 ± 0.058 to 102.92 ± 0.331 mg GAE/g extract. The flavonoids contents of the extracts in the

Table 1: Total Polyphenol and Flavonoid of the extracts.

Extract	Total polyphenol content (mg/g GAE)	Total flavonoid content (mg/g QE)
EHD	19.55 ± 0.058	0
EEAD	90.62 ±0,101	1.004 ± 0,016
EED	102.92 ± 0.331	1.88 ± 0.003

Table 2: Antioxidant activity of hexane, ethyl acetate, and ethanol extract of *Durio zibethinus* Murr

Extract	β-Caroten-Linoleic Acid Assay			DPPH Scavanging Acivity		
	Concentration (µg/ml)	Percent Inhibition (%)	IC_{50} (µg/ml)	Concentration (µg/ml)	Percent Inhibition (%)	IC_{50} (µg/ml)
EED	100	38.521	166.832	1	8.702	11.213
	200	58.495		5	25.926	
	300	64.721		10	44.236	
	400	75.356		15	69.330	
	500	80.804		20	83.354	
EEAD	100	40.337	139.533	25	13.733	83.951
	200	61.349		50	29.713	
	300	69.649		75	45.277	
	400	80.285		100	61.506	
	500	84.436		125	74.365	
EHD	100	25.628	273.58	500	36.288	541.279
	200	40.736		600	51.144	
	300	52.749		700	58.718	
	400	68.953		800	67.000	
	500	80.012		900	72.742	

Table 3: α-Glucosidase Inhibitory Activity of *Durio zibethinus* Murr. Extracts.

Extract	α-Glucosidase Inhibitory Activity		
	Concentration (µg/ml)	Percent Inhibition (%)	IC_{50} µg/ml
EED	5	67.012	3.346
	4	63.629	
	3	59.093	
	2	24.567	
	1	8.997	
EEAD	250	75.988	23.693
	200	74.241	
	150	73.283	
	100	72.701	
	50	55.514	
EHD	250	86.874	119.84
	200	76.232	
	150	60.124	
	100	31.014	
	50	22.029	

terms of standard quersetin equivalents (QE) were between 0 to 1.88 ± 0.003 mg QE/g extract. Among the tested extracts, EED has the highest amount of total phenolics and flavonoids compound.The phenolics compound contained of hydroxyl groups which made it soluble in higher polarity solvent such as ethanol.[15] The hexane extract showed the lowest content of polyphenol (19.55 GAE mg/g dw) and flavonoid (failed to extract flavonoid content 0 mg QE/g dw) compounds due to its low polarity.

Polyphenols is a major antioxidant compound in plants.Based on the high phenolics and flavonoids content of the extracts, they could be a potential antioxidant resource. The EED extract has the best antioxidant activity against DPPH with IC_{50} of 11.21µg/ml compared to standard ascorbic acid which exhibited 50% inhibition at the concentration of 6.56 µg/ml because of the high phenolics and flavonoids content in EED allow it to donate electron such as hydroxyl group to the reactive species to form a non radical DPPH-H form in the homogenous system.[13,16]

The EEAD extracts also revealed a quite potential antioxidant with IC_{50} of 83.951 ppm followed with hexane extract 541.28 ppm. The hexane extract showed the lowest antioxiant activity because it contained of low poliphenolic and flavonoid compound. The same pattern also shown from the determination of antioxidant activity of the *Durio zibethinus* Murr extacts using β-carotene-linoleic acid assay. The three extracts exhibited an antioxidant capacity by protecting oxidation of linoleic acid in β-carotene emulsion to form hidroperoxides which would caused discoloration of β-carotene. The high absorbace of β-carotene emulsion after 120 min of incubation means the high antioxidant capacity of the extract[13]. Poliphenol compounds exhibited a high benefit for protection of lipid peroxidation as have been studied by Dey[17] showed that poliphenol from grapes decreased the liver triglicerol of a rat fed high-fat-high-sucrose diet.

The chemical component in *Durio zibethinus* Murr are promised as the source of α glcosidase inhibitor because it contained of high of polyphenols and revealed a high antioxidants capacity. In this study, we investigate the activity of different extracts from *Durio zibethinus* Murr. It was found that the extracts showed different inhibitory activity against α glucosidase enzyme which ranged between 3.346 to 119.84 µg/ml. The EED exhibit the highest activity to inhibit α glucosidase enzyme with IC_{50} 3.346 µg/ml followed with EEAD with IC_{50} 23.693 µg/ml and EHD 119.84 µg/ml.

In this study, the high polyphenol content of the extracts showed potential activity to be usefull for diabetes treatment by two possible mechanisms which were inhibited the α-glucosidase enzyme to decrease and control post prandial hyperglycemia and protect hyperglycemia mediated hepatic injury by decrease generation of superoxide and prevent lipid peroxidation as their capacity to prevent lipid peroxidation *in vitro*.[17]

CONCLUSION

In conclusion, this study clearly resulted that *Durio Zibethinus* Murr. has high potency as antioxidant activity against free radicals *in vitro*. The antioxidant activity of this potent medicinal plant may be attributed to

its compounds potency to scavange the free radicals as well as to inhibit lipid peroxidation. This medicinal plants also has a high potency as antidiabetic agents based on its high power to inhibit the α-glucosidase enzyme *in vitro*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

EHD: Hexane extract of *Durio zibethinus* Murr. root; **EEAD:** Ethylacetate extract of *Durio zibethinus* Murr. root; **EED:** Ethanol extract of *Durio zibethinus* Murr. Root; **DPPH:** 1,1-Diphenyl 2-picrylhydrazine; **GAE:** Gallic acid equivalent; **QE:** Quercetin equivalent; **UV-Vis:** Ultraviolet-visible.

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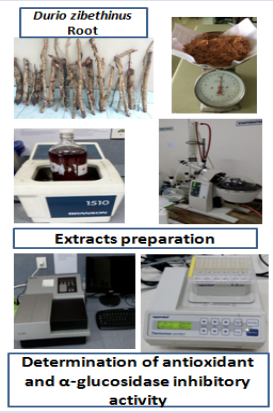
GRAPHICAL ABSTRACT



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SUMMARY

- Active natural products from medicinal plants are good sources for the antioxidant and antidiabetic agent. Natural antioxidants protect human health against oxidative stress and free radicals which cause degenerative disease such as diabetes. One approach to treat diabetes is to reduce gastrointestinal glucose absorption through the inhibition of the α-glucosidase enzyme. In this study, we used *Durio zibethinus* Murr roots which taken from Wotu, South Sulawesi, Indonesia. We make three different root extracts from *Durio zibethinus* Murr root by gradual extraction system. The extracts were examined for their DPPH radical scavenging activity, antioxidant activity using β carotene-linoleic acid assay, and their α-glucosidase inhibitory activity by standar method. The measurements were done using Biotek Microplate Reader. Additionally, we were also determined their Total polyphenols and total favonoids quantitatively using spectofotometer UV-Vis. The root extracts of *Durio Zibethinus* Murr were effectively scavenged DPPH radicals in varied rate. The hexane (EHD), ethyl acetate (EEAD), and ethanol (EED) extracts revealed DPPH radical scavenging activity with IC_{50} of 541.28, 83.95 and 11.24 µg/ml respectively and their β-carotene-linoleic acid assay showed activity with IC_{50} of 0, 139.53, and 166.83 µg/ml, respectively. *In vitro* assay of the α-glucosidase inhibitory activity of the EHD, EEAD, and EED extracts showed an IC_{50} of 119.84, 23.69, and 3.35 µg/ml, respectively. In this present study, we found that ethanol extract revealed the most active antioxidant and the highest inhibitory activity against α-glucosidase enzyme. The total phenolics contents of the EHD, EEAD, and EED extracts were 19.55 ± 0.058; 90.62 ±0,101; 102.92 ± 0.331 mg GAE/g extracts. The total flavonoid contents of the EHD, EEAD, and EED extracts were 0; 1.004 ± 0,016; 1.88 ± 0.003 mg QE/g extracts. The ethanol extract have the highest polyphenols and flavonoids content than that of other extracts and in accordance with its antioxydant and antidiabetes activity. The high polyphenol content of the extracts showed potential activity to be usefull for diabetes treatment by two possible mechanisms which were inhibited the α-glucosidase enzyme to decrease and control post prandial hyperglycemia and protect hyperglycemia mediated hepatic injury by decrease generation of superoxide and prevent lipid peroxidation as their capacity to prevent lipid peroxidation *in vitro*. This study proofs the medicinal potencies of *Durio zibethinus* Murr. root extracts.

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