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

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**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 conducted 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 (EH), ethyl acetate (EAA), and ethanol (EED) extracts revealed DPPH radical scavenging activity with IC₅₀ of 541.28, 83.95 and 11.24 μg/ml respectively and their β-carotene-linoleic acid assay showed activity with IC₅₀ of 273.68, 139.53, and 166.83 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.

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

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 constituents determination
Total Phenolic Content
Total phenolic content of the extracts were examined by Folin-Ciocalteu method with slight modification. The extract were prepared in methanol and 100 µl of each concentration in 5 ml volumetric flask was added with 2.5 ml Folin Ciocalteu’s reagent (7.5%). The mixtures were vortexed and incubated at room temperature for 10 min. After incubation, 2 ml of NaOH 1% was added and the mixture was vortexed. 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 spectrophotometer (UV 1800 Shimadzu, Japan). Gallic acid was used as standard. The absorbance was measured at 431 nm with a UV/Vis spectrofotometer (Shimadzu). All measurements were done in triplicate. The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration. All tests were performed in triplicate.

Total Flavonoid Content
Total flavonoid content was determined using aluminium-chloride colorimetric assay. Briefly, 1 ml of extract solution in suitable solvent was mixed with 1.5 ml ethanol, 100 l of AlCl₃ (10%) and 100 l 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 spectrophotometer (Shimadzu). All measurements were done in triplicate.

DPPH Radical Scavenging Assay
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.


dpph scavenging effect (%) or percent inhibition = \frac{A_0 - A_t}{A_0} \times 100

Where A₀ was the absorbance of control reaction and Aₜ 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 with slight modification. Three milliliter of β-carotene solution (0.2 mg/ml in chloroform) was pipetted 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 shaken 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:

\[
AA = \left(1 - \left(\frac{A_0 - A_{t0}}{A_0 - A_{t1}}\right)\right) \times 100
\]

Where AA is antioxidant activity, A₀ and Aₜ are the absorbance of blank and sample at the initial time of incubation respectively. While A₀ and Aₜ are absorbance of blank and sample at 120 min of incubation.

α-Glycosidase inhibitory assay
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 14 l 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 mM Sodium carbonate. The liberated p-nitrophenol was determined at 405 nm using Biotek Microplate Reader. The % inhibition rates were calculated using the formula,

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

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.

### Table 1: Total Polyphenol and Flavonoid of the extracts.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total polyphenol content (mg/g GAE)</th>
<th>Total flavonoid content (mg/g QE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHD</td>
<td>19.55 ± 0.058</td>
<td>0</td>
</tr>
<tr>
<td>EEAD</td>
<td>90.62 ±0.101</td>
<td>1.004 ± 0.016</td>
</tr>
<tr>
<td>EED</td>
<td>102.92 ± 0.331</td>
<td>1.88 ± 0.003</td>
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Table 2: Antioxidant activity of hexane, ethyl acetate, and ethanol extract of *Durio zibethinus* Murr.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration (µg/ml)</th>
<th>Percent Inhibition (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
<th>Concentration (µg/ml)</th>
<th>Percent Inhibition (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
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<td>100</td>
<td>38.521</td>
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<td>50</td>
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<td></td>
<td>200</td>
<td>58.495</td>
<td>15</td>
<td>100</td>
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<tr>
<td>EED</td>
<td>300</td>
<td>64.721</td>
<td>166.832</td>
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<td>75.356</td>
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<td>83.354</td>
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<td>200</td>
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Table 3: α-Glucosidase Inhibitory Activity of *Durio zibethinus* Murr. Extracts.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration (µg/ml)</th>
<th>Percent Inhibition (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
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<td>250</td>
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<td></td>
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<tr>
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<td>23.693</td>
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<td></td>
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<tr>
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<tr>
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</table>

The chemical component in *Durio zibethinus* Murr. are promised as the source of a glucosidase 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 exhibited the highest activity to inhibit a glucosidase enzyme with IC<sub>50</sub> of 3.346 µg/ml followed with EEAD with IC<sub>50</sub> 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 useful 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.15

**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

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. 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<sub>50</sub> of 11.21 ppm followed with hexane extract 541.28 ppm. The hexane extract showed the lowest antioxidant activity because it contained of low polyphenolic and flavonoid compound. The same pattern also shown from the determination of antioxidant activity of the *Durio zibethinus* Murr extracts 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 absorbance of β-carotene emulsion after 120 min of incubation means the high antioxidant capacity of the extract. Poliphenol compounds exhibited a high benefit for protection of lipid peroxidation as have been studied by Dey showed that poliphenol from grapes decreased the liver triglicerol of a rat fed high-fat-high-sucrose diet.

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 EED extracts also revealed a quite potential antioxidant with IC<sub>50</sub> of 83.951 ppm followed with hexane extract 541.28 ppm. The hexane extract showed the lowest antioxidant activity because it contained of low poliphenolic and flavonoid compound. The pattern also shown from the determination of antioxidant activity of the *Durio zibethinus* Murr extracts 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 absorbance of β-carotene emulsion after 120 min of incubation means the high antioxidant capacity of the extract. Poliphenol compounds exhibited a high benefit for protection of lipid peroxidation as have been studied by Dey. showed that poliphenol from grapes decreased the liver triglicerol of a rat fed high-fat-high-sucrose diet.

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**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 scavenge 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.

ACKNOWLEDGEMENT

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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.

REFERENCES


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 gradient 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 standard method. The measurements were done using Biotek Microplate Reader. Additionally, we were also determined their Total polyphenols and total flavonoids quantitatively using spectrophotometer 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 IC₅₀ of 541.28, 83.95 and 11.24 µg/ml respectively and their β-carotene-linoleic acid assay showed activity with IC₅₀ of 0, 139.83, and 166.83 µg/ml, respectively. In vitro assay of the α-glucosidase inhibitory activity of the EHD, EEAD, and EED extracts showed an IC₅₀ 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 antioxidiant 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.