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Brief Communication

Phytochemical Screening and Antidiabetic Activity of N-hexane, Ethyl acetate and Water Extract from Durian Leaves *(Durio zibethinus L.)*

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ABSTRACT

The leaf of Durian *(Durio zibenthinus L.)* contain flavonoids and steroids in ethanol extract. Natural flavonoids have an important role in the prevention of diabetes and its complications and are able to provide good effects in the fight against *diabetes mellitus*, either through the ability to reduce glucose absorption and improve glucose tolerance. The purpose of this research is to know the activity of antidiabetic extract of n-hexane, ethyl acetate and water from durian leaf ethanol extract. The results of phytochemical screening screen showed that durian leaf contains chemical compounds of flavonoids, steroids, and glycosides. The treatment was gradual to obtain durian leaf extract i.e. with 96% ethanol, then partitioned gradually with n-hexane and ethyl acetate. Therefore, the extract of n-hexane, ethyl acetate and water is obtained. All three extracts were tested activity antidiabetic with inhibition method of α-glucosidase enzyme activity. The inhibitory results of α-glucosidase enzyme activity showed IC_{50} values for n-hexane, ethyl acetate and water extracts of 9.79 µg/mL; 38.18 µg/mL and 35.83 µg/mL. The results show that the polar chemical component of the durian leaf is potential as an antidiabetic.

Keywords: Phytochemistry, *Diabetes mellitus,* Antidiabetic, Extraction, Flavonoids, *Durio zibenthinus L.*

INTRODUCTION

Diabetes mellitus is a metabolic disorder caused by a lack of insulin in a person's body. The lack of insulin in a person's body sugar

(glucose) consumed by the body cannot be processed perfectly. This situation causes sufferers to experience hyperglycemia or excess blood sugar. In diabetes, excessive blood sugar levels can cause long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels[1]. How to treat *diabetes mellitus* is one of them by inhibiting the α-glucosidase enzyme. The enzyme has a role in converting carbohydrates to sugar (glucose) can be returned to normal limits[2]. Durian *(Durio zibethinus L.)* is one of the Malvaceae family plants which has antidiabetic activity on durian skin ethanol extract[3]. Phytochemical screening on Durian fruit contains the chemical compound flavonoids, polyphenols[4]. In the methanol extract durian bark contains flavonoids, alkaloids, tannins, terpenoids, and steroids[5]. The aim of the study was to determine the chemical compounds of durian leaves and to test antidiabetic activity so that it could be developed as a natural medicine.

MATERIALS AND METHODS

The material used in the study is. Ethanol 96%, n-hexane, ethyl acetate, 1% DMSO, 0.1 M phosphate buffer and 0.01 M, 0.5 mM p-nitrophenyl-α-D-glucosidase substrate, 0.04 α-glucosidase enzyme / mL, Na_2CO_3 0.2 M, distilled water.

Research Methods. Sample drying cabinet, simplicia grinder, set of distillation apparatus, rotary evaporator, Ultraviolet-Visible Spectrophotometer (Hitachi U-3900H), micropipette, tip, measuring cup, measuring flask, analytic scale, incubator (Jouan), and a set of glass tools.

Phytochemical Screening. Identification flavonoids. 10 g each extract, dissolved 100 mL hot water, boiled for 5 min and filtered in hot condition, into 5 mL of filtrate added 0.1 g of magnesium powder and 1 mL of concentrated hydrochloric acid and 2 mL of amyl alcohol, shaken and allowed to separate. Flavonoids positive if there is a red or yellow or orange color in the amyl alcohol layer[6] Identification saponins. The extract was weighed as much as 0.5 g and put into a test tube, then added 10 mL of hot water, cooled, then shaken vigorously for 10 minutes. If foam is formed as high as 1-10 cm that is stable not less than 10 min and the foam does not disappear with the addition of 1 drop of 2N hydrocloride acid indicating the presence of saponins.[7]

Identification tanins. A total of 1 g of extract was weighed, along with 10 mL of distilled water and then filtered, the filtrate was diluted with water until colorless. The solution was taken as much as 2 mL and added 1-2 drops of iron (III) chloride reagent. If there is a blue or blackish green color indicates the presence of tannins.

Identification alkaloids. The weighed extract was 0.5 g then added 1 mL of 2N hydrochloride acid and 9 mL of distilled water, heated on a water bath for 2 min cooled and filtered. The obtained filtrate is used for alkaloid tests. Three test tubes are taken, then put 0.5 mL of filtrate in to it. In each test tube added 2 drops mayer reagents, 2 drops Wagner reagent, Dragendorff reagent. Alkaloids are positives if there is sediment or turbidity in two of the three experiments above.

Identification glycosides. A total of 3 gram extract added 30 mL of ethanol : water (7:3) refluxed for 10 min cooled and filtered. Then 20 mL of filtrate was taken with 25 mL of distilled water and 25 mL of lead (II) acetate 0.4 M, shaken, allowed to stand for 5 min then filtered. The filtrate was added 20 mL of a mixture of chloroform and isopropanol (3:2), repeated three times. Then evaporated at a temperature of not more than 50^0C. the rest is dissolved in 2 mL of methanol. The remaining solution is used for experiments: as much as 0.1 mL if the experimental solution is put into a test tube and evaporated over a water bath. The remaining 2 mL of water and added 5 drops of Molish reagent. Then added 2 mL of sulfuric acid through the tube wall with slowly. Forming a purple ring at the boundary of the two liquids, indicating the presence of a sugar bond.

Identification steroids/terpenoids. The extract was weighed as much as 1 g macerated with 20 mL of n-hexane for 2 h filtered. The filtered was evaporated in a vaporized plate and the remaining Liebermann-Burchard reagent was added through the cup wall. If a purple or red color turns blue or blue or green blue indicates the presence of triterpenoids/steroids.

Inhibition of the enzyme α-glucosidase. Tests were carried out based on[8] with a phosphate buffer 0.1 M pH 7.0 as much as 50 µL added 25 µL

0.5 mM 4-nitrophenyl α-D-glucopyranose. The mixture was brokububated for 5 min at a temperature of 37^{0}C and then added 10 µL of the tested sample. Extracts were made with varying concentrations of 5 ppm, 10 ppm, 25 ppm, 50 ppm, and 100 solutions were added 250 µL of α-glucosidase solution, incubated for 30 min at 37^{0}C. The solution is terminated with the addition of 1000 µL of 0.2 M Na_2CO_3 solution. The inhibition of the extract is measured on λ 410 nm with microplate reader. The experiment was carried out in triplo

RESULTS AND DISCUSSION

The leaves of durian 7 kg, drying in room temperature, and cut small, then made powder. We got the powder of leaves durian 4,8 kg. The powder was maceration with ethanol 96%. We got the ethanol extract 185,2 gram. Phytochemical screening results is done in maceration for 2 x 24 hours. Extraction is filtered to separate the filtr ate from the residue. The residue is remacerated until the obtained filtrate does not contain flavonoids. The maceration filtrate was evaporated using a rotary evaporator to obtain ethanol extract of Durian leaf [9]. In ethanol extract, n-hexane and water were added (1:1), then the extract was filtered, and the n-hexane filtrate was evaporated. Ethyl acetate is added to the water extract, then filtered and evaporated from ethyl acetate and water extraction. Then each extract, namely n-hexane, ethyl acetate, and water extract, was screened for phytochemical screening and antidiabetic testing. Phytochemical screening screening for Durian *(Durio zibethinus L.)* leaves includes examination of secondary metabolite compounds such as alkaloids, saponins, flavonoids, tannins, glycosides, and terpenoids/steroids[7] described in Table 1.

Fig. 1. Leaves of Durian

Table 1: Phytochemical Screening of Durian *(Durio zibethinus L.)* leaves

No	Metabolite	Ethanol	n-Hexane	Water	Ethyl acetate
1	Alkaloids	-	-	-	-
2	Saponins	-	-	-	-
3	Flavonoids	+	-	-	+
4	Tannins	-	-	+	-
5	Terpenoids/Steroids	+	+	-	+
6	Glycosides	+	-	+	+

Antidiabetic Activity

Preliminary test and inhibition of the enzyme α-glucosidase is the hydrolysis of the enzyme against p-nitrophenyl-α-D-glucopyranoside to p-nitrophenol which gives a yellow color[10]. The enzyme inhibitory activity test was carried out by spectropotometry method at a wavelength of 410 nm. This test was carried out at different extract concentrations to determine the effect of the concentration of each extract on IC_{50} inhibitory power, the greater the percent value indicated, the greater the inhibition of the enzyme, and vice versa. IC_{50} values showed the ability of the extract to inhibit enzyme activity by 50%. The IC_{50} value <50 µg/mL is very strong, if the value of 50-100 µg/mL is said to be strong, if the value of 100-150 µg/mL is said to be weak, the value of 150-200 µg/mL is said to be very weak if the smaller IC_{50} value indicates activity higher inhibition, and vice versa. The results of this study indicate that the IC_{50} value of each extract of n-hexane, ethyl acetate and water is 35.83 µg/mL; 38.18 µg/mL; and 9.79 µg/mL. This shows that water extract has the potential as a source of active chemical compounds for diabetic drugs which are polar compounds.

Table 2: Test results for Antidiabetic Activity of n-Hexane, Ethyl acetate, and Water extract of Durian *(Durio zibethinus L.)* leaves with inhibition method of α-glucosidase enzyme activity

Extracts	Concentration (ppm)	Inhibition (%)	IC_{50} (ppm)
Water Extract	5	227,882	97,889
	10	522,063	
	25	565,974	
	50	610,317	
	100	625,027	
Ethyl acetate Extract	5	227,666	38.1772
	10	271,793	
	25	316,136	
	50	632,057	
	100	668,937	
	-Hexane Extract	5	139,411
358,300			
	10	389,682	
	25	433,809	
	50	595,392	

CONCLUSION

Phytochemical screening results showed that secondary metabolites such as flavonoids, terpenoids/steroids, glycosides, existed in *Durio zibethinus L*. durian leaves. The results showed that antidiabetic activity in Durian leaf water extract was very strong because IC<50 ppm was 9.7889 ppm compared with n-hexane and ethyl acetate extract of Durian leaves.

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