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Characterization and Antidiabetic Potential of Durian Leaf (Durio *zibethinus* Linn.) Ethyl Acetate Extract

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Abstract: Durian leaves (*Duiro zibethinus* Linn.) are one of the plants whose leaves are used as a fever reducer (antipyretic). The aim of this research is to isolate natural ingredients using natural ingredient extraction techniques by maceration and identify steroid compounds from durian leaves that have anti-diabetic potential. Isolation was carried out through several stages, namely maceration, fractionation, subfractionation using column chromatography, purification using preparative TLC, and testing the purity of the isolate using TLC and determining the melting point of the isolate. The antidiabetic potential of the isolation was carried out using the α-glucosidase enzyme inhibition method. The chemical structure of the isolate was characterized using IR and UV-Vis spectroscopy. Based on IR spectroscopy analysis, the isolation has C=C, OH, O-C functional groups, C-H aromatic functional groups. UV-Vis spectroscopy data shows that there are maximum peaks at 207 nm and 247 nm, meaning that the isolated steroid compound has unconjugated double bonds. Based on spectroscopic data and comparison with reference compounds, it could be identified that the isolate obtained was β-sitosterol.

Kata kunci: durian leaves, *durio zibethinus* Linn., antidiabetic, steroid, β-sitosterol

***Abstract*:** *Daun durian (Duiro zibethinus Linn.) merupakan salah satu tanaman yang daunnya digunakan sebagai penurun demam (antipiretik). Tujuan penelitian ini adalah untuk mengisolasi bahan alam dengan teknik ekstraksi bahan alam dengan cara maserasi serta identfikasi senyawa steroid dari daun durian yang berpotensi sebagai antidiabetes. Isolasi dilakukan melalui beberapa tahap yaitu maserasi, fraksinasi, subfraksinasi menggunakan kromatografi kolom, pemurnian menggunakan KLT preparatif, dan pengujian kemurnian isolat menggunakan KLT serta penentuan tititk leleh isolat. Potensi antidiabetes dari isolasi dilakukan dengan menggunakan metode penghambatan enzim α-glukosidase. Isolat karakterisasi struktur kimianya menggunakan spektroskopi IR dan UV-Vis. Berdasakan analisis spektroskopi IR, isolasi memiliki gugus fungsi C=C, OH, O-C, gugus fungsi C-H aromatik. Data spektroskopi UV-Vis menunjukkan adanya puncak maksimum pada 207 nm dan 247 nm berarti bahwa senyawa steroid hasil isolasi mempunyai ikatan rangkap yang tidak berkonjugasi. Berdasarkan data spektroskopi dan perbandingan dengan senyawa referensi dapat diidentifikasi bahwa isolat yang diperoleh adalah β-sitosterol.*

Keywords: *daun durian, durio zibethinus Linn., antidiabetes, steroid, β-sitosterol*

INTRODUCTION

Secondary metabolite compounds contained in plants are bioactive substances that contain chemicals in plants that are interpreted as self-defense mechanisms against environmental threats, so that plants can be used as medicinal ingredients for various diseases (Nugroho 2017). Indonesia's biodiversity potential is the second largest in this world which gives birth to chemodiversity (chemical diversity) is secondary metabolite compounds (Achmad 2000). Many of them have biological activities that can treat various diseases, one of which is treating diabetes mellitus due to deficiency insulin. Studies shows that the ethanol extract of durian fruit peel has activity *in-vitro* antihyperglycemic (Roongpisuthipong *et al.*1991). Durian leaves (*Durio zibethinus*) is one of the plants that is empirically used as an alternative to treat fever (antipyretic) for children. Durian plants belonging to the Bombacaceae family contain secondary metabolites, namely flavonoids, tannins, steroid, glycosides (Aruan dkk. 2019). Several studies have reported that *Durio zibethinus* L. leaves have bioactivity as an antidiabetic in ethyl acetate extract (Aruan *dkk*. 2019), as antihyperuricemia (Sonia dkk. 2020), has toxicity and antioxidant activity in aqueous extracts,

ethyl acetate extract, *n*-hexane (Aruan 2019), has antibacterial activity in aqueous extracts, ethyl acetate extract, and ethanol (Rizki dkk. 2022). The aim of this study was to isolate and characterize the antidiabetic potential of durian leaves (*Durio zibethinus* L.).

MATERIALS AND METHODS

Materials and Tools

The chemicals in this research at a technical level include: 96% ethanol (Brataco), *n*-hexane (Brataco), ethyl acetate (Brataco), aquadest, silica gel Merch GF254, methanol (Brataco), Liebermann-Burchard reagent (LB) for testing steroids, *α*-glucosidase enzymes.

The tools used to carry out this research are as follows: the maceration process includes an analytical balance, glass jar 5L (kombucha), a set of glassware (IWAKI pyrex), chromatography gravity column, chamber, thin layer chromatography (TLC) was perfomed on silica gel G (10 to 40 mesh, Merck), spektrofotometer infra red (IR, Shimadzhu FTIR-820), UV spectrophotometer (UV, Milton Roy Spectronic-300Array), UV lamps (Camac UV-cabinet II), The ^{1}H and ^{13}C NMR spectra were recorded $CDCl_3$ using TMS as internal standard on Bruker AV-300 FTNMR spectrometer at 300 and 75 MHz.

Sample Preparation and Test for Steroid

The process of making simplicia is that first the fresh durian leaves are cleaned of dirt, weighed, then dried in a drying cupboard and not exposed to direct sunlight, then a phytochemical test is carried out. The steroid test was carried out by taking 10 grams of sample and then adding methanol for extraction. The methanol extract obtained was extracted with diethyl ether. The residue that was not dissolved in diethyl ether was shaken vigorously. The solution was hydrolyzed with HCl and filtered. The ether extract was tested with Liebermann Burchard reagent. Green or blue color indicates the presence of steroids.

Isolation of Steroid Compounds and Purification of Isolates

Total ethyl acetate extract was column chromatographed with silica gel 60 as the stationary phase and *n*-hexane: ethyl acetate as the mobile phase which was determined from the results of the thin layer chromatography or TLC test. Each fraction is collected. Each fraction obtained was analyzed by TLC and the fractions that had the same stain pattern were combined. Then chromatography was performed until pure isolate was obtained which was indicated by the appearance of a single stain from TLC analysis and preparative TLC was performed. The pure isolates obtained were collected using UV and FTIR spectra data.

RESULT AND DISCUSSION

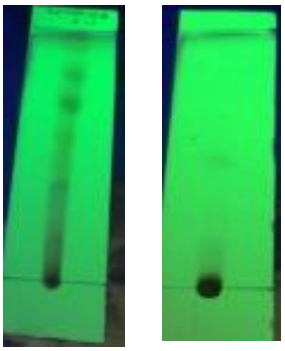
Sample Preparation

Durian leaf powder as much as 4.8 kg was macerated at room temperature using 96% ethanol solvent. The maceration process was carried out for 48 hours with remaceration treatment up to 7 times for the residue, so that all components are expected to be extracted into ethanol solvent. Then the extract was collected and the solvent was evaporated using a rotary evaporator to obtain a concentrated ethanol extract of 185.2 g. The concentrated ethanol extract is continued to the partitioning stage using *n*-hexane, ethyl acetate, and water solvents which aim to separate the non-polar, semi-polar, and polar chemical components.

Sample Preparation

Isolation of steroid compounds was carried out after the *n*-hexane, ethyl acetate, and water extracts were subjected to phytochemical screening to determine the presence of secondary metabolites contained in each extract. The partition results obtained *n*-hexane extract, ethyl acetate extract, and water extract. Each extract contains flavonoids, glycosides, and steroids. Each extract was tested for antidiabetic and the inhibition concentration value of 50 (IC_{50}) resulted in an inhibition value of the ethyl acetate extract of 38.83. IC_{50} shows the ability of the sample to inhibit activity enzyme by 50 percent, so the value is smaller IC_{50} shows higher inhibitory activity, and on the contary. That $IC_{50} < 50$ μg/mL is very strong, if it is 50-100 μg/mL, while IC_{50} is 100-150 μg/mL, weak if IC_{50} is 150-200 μg/mL, and very weak if the IC_{50} value is >200 μg/mL (Mardawati 2008). The ethyl acetate extract is continued to the isolation and purification stage of the ethyl acetate extract. Before the ethyl acetate extract is fractionated, optimization of the solvent that will be used to isolate the extract is carried out. The eluent used to elute the extract was *n*-hexane:ethyl acetate 10:1 and 2:1. The results can be seen in Figure 1.

The results of the TLC analysis showed that the eluent *n*-hexane:ethyl acetate (2:1) gave too high an elution compared to *n*-hexane:ethyl acetate (10:1) which was not too high and gave better results with spots that did not accumulate so that the spots see clearly. The results of TLC analysis showed that the mobile phase used for first column chromatography was *n*-hexane:ethyl acetate (10:1). Separation of ethyl acetate extract by column chromatography using silica gel 60 stationary phase (70-230 mesh) with *n*-hexane:ethyl acetate as eluent mobile phase (10:1; 8:1; 6:1; 4:1; 2:1 ; 1:1) and obtained 131 fractions and accommodated in bottles with a volume of less than 50 mL. Then evaporated and analyzed by TLC using silica gel GF_{254} stationary phase and *n*-hexane:ethyl acetate mobile phase (5:1). The fractions having the same Rf were combined to obtain 8 simple fractions, namely the F1-F8 fractions. Then F1-F8 were tested for antidiabetic activity.



a) b)

Figure 1. TLC analysis for 2:1 and 10:1 eluent chromatography chromatography mobile phase analysis of *n*-hexane:ethylacetate a) 2:1 and b) 10:1

Table 1. Fractionation results and weights from first column chromatography

No	Fraksi	Vial	Bobot (g)	Rf
1	F1	1-7	0,43	0,9
2	F2	8-18	0,29	0,8
3	F3	19-24	0,2	0,75
4	F4	25-37	0,34	0,73
5	F5	38-68	0,63	0,67
6	F6	69-77	0,4	0,63
7	F7	78-115	1,89	0,55
8	F8	116-131	4,35	0,50

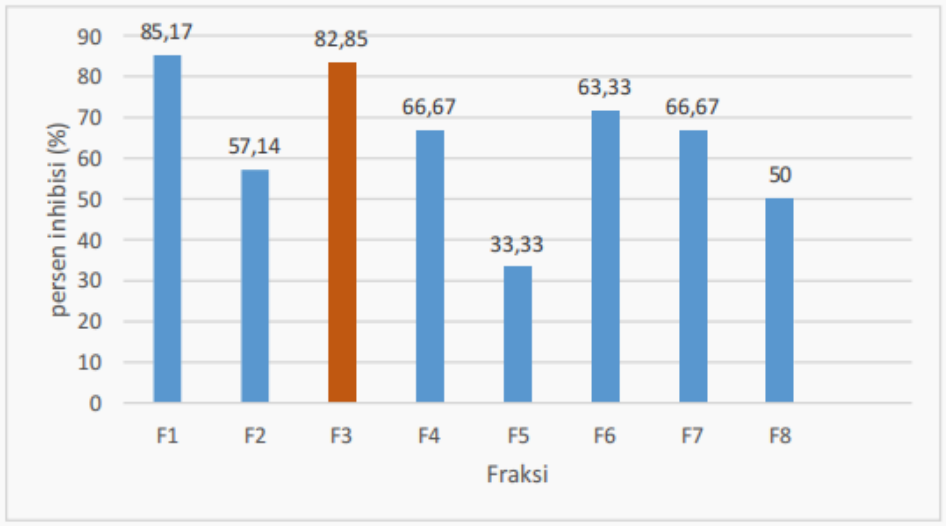


Figure 2. Diagram of percent inhibition values of α-glulcosidase enzyme fraction result of first column chromatography

Test of α-glucosidase Enzyme Inhibition Activity Against Fractions

In the antidiabetic activity test using the *α*-glucosidase enzyme inhibition method in vitro for the F1-F8 fraction group as a result of column chromatography with a sample solution concentration of 100 ppm. The results of the column chromatography of the F3 fraction had the activity of inhibiting the *α*-glucosidase enzyme *in vitro* with an inhibition percentage of 82,8%. The results of the first column chromatographic fractionation for the ethyl acetate fraction of durian leaves were as much as 200 mg of the green F3 fraction which was thought to be a steroid group compound.

Separation and Fraction Purification Stages

A total of 200 mg of the fraction (F3) was separated using column chromatography with a column length of 40 cm and a diameter of 2,5 cm. The results of the TLC fractions has an Rf value of 0,7 weighing 101 mg. Continued for preparative TLC with weight of 81 mg was obtained, white powder, melting point 132-134^{0}C. The isolate was already pure was characterized by UV-Vis and FTIR, NMR, and LCMS.

Analysis of Isolate by Ultra Violet Spectroscopy

Isolate analyzed by ultra violet spectroscopy showed the maximum wavelength at 207 and 247 nm where π-π* electron transitions occurred indicating the presence of an unconjugated C=C bond. The spectrum of isolate can be seen in Figure 3.

Figure 3 results of the UV spectrum of isolate carried out at a wavelength of 200-400 nm, showing that there are maximum peaks found at wavelengths of 207 nm and 247 nm with an absorption of 0.937 meaning that this isolated steroid compound has an unconjugated double bond and a wavelength of 247 nm.

Analysis of Isolate with FTIR

The results of the IR spectrum analysis of isolate shows a broad absorption in the wave number region 3410 cm-1 from the O-H group (3200-3550 cm-1). This group is reinforced by the presence of absorption at wave number 1371.39 cm-1 indicating absorption from the O-C group (1330-1420 cm-1) and wave number 1016.49 cm-1 indicating the presence of C-H (990-1060 cm-1). The absorption band at the wave number indicates the OH group. Absorption with strong intensity at wave numbers 2864.29-2945.20 cm-1 is the absorption of the C-H group from CH_2 (alkanes, 2850-3000 cm-1). This group is strengthened by absorption at wave number 1454 cm-1 indicating the absorption of the C-H group from CH_2 (1450-1470 cm-1). At the absorption wave number of 1676.14 cm-1, it shows the absorption of the C=C group (1620-1680 cm-1) (Eaton & Cole 1964).

Analysis of Isolate with NMR ^{1}H dan ^{13}C

Nuclear magnetic resonance (NMR) spectral data analysis is based on Silverstein & Webster (1998). Resonance spectra proton magnetic (^{1}H-NMR) and carbon magnetic resonance spectra (^{13}C-NMR) obtained by dissolving the sample in deutered-chloroform ($CDCl_3$) in 0,5 mL each in NMR tubes (5 mm). Spectra recorded Fon spectrometer Jeol 500 (^{1}H-NMR at 500 MHz and ^{13}C-NMR at 125 MHz).

Proton nuclear magnetic resonance (^{1}H-NMR) spectrum of isolate show the types of protons and the number of protons that are found in isolate. The methyl proton appears in the chemical shift region (δH) 0,68 (s, H-29); 0,84 (d, J=5,5 Hz, H-26); 0,86 (d, J=5,5) Hz, H-27); 0,93 (d, broad, H-21) and 1,00 (s, H-19). There is a methylene proton (-CH_2-) as multiplets in the high magnetic field region δH 0,68~3,55 ppm, where δH 3,55 is the proton for the methane group CH-OH (H-3). The presence of protons at δH 5,36 (t, 1H) indicates the presence of protons in the region low magnetic field for =CH- on C-6.

Carbon-13 NMR (^{13}C-NMR) is an application of nuclear magnetic spectroscopy (NMR) carbon resonance. ^{13}C-NMR detects only the ^{13}C isotope of carbon (the number of C atoms) either as C-methyl,

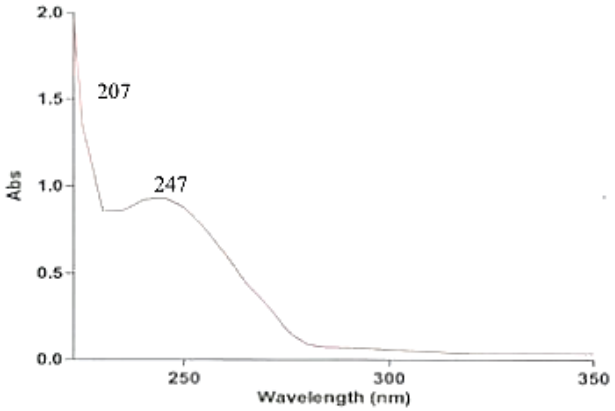


Figure 3. UV spectrum of isolate

Table 3. Proton and carbon chemical shifts in isolate compared to β-sitosterol compounds

Position	C isolate	DEPT	H isolate
1	37,43	CH_2	1,83 (m)
2	31,85	CH_2	1,56 (m)
3	71,99	CH	3,52 (t)
4	42,49	CH_2	2,28
5	140,94	C	-
6	121,92	CH=	5,36
7	32,09	CH_2	1,99
8	32,09	CH	1,56
9	50,30	CH	1,56
10	36,69	C	-
11	21,26	CH_2	1,56
12	39,96	CH_2	1,56
13	42,51	C	-
14	56,95	CH	1,56
15	24,50	CH_2	1,56
16	28,44	CH_2	1,83
17	56,23	CH	1,56
18	12,05	CH_3	0,68 (s)
19	20,01	CH_3	1,00 (s)
20	36,33	CH	1,56 (m)
21	18,96	CH_3	0,93 (d)
22	34,12	CH_2	0,92 (m)
23	26,23	CH_2	1,17 (m)
24	46,02	CH	1,37 (m)
25	29,32	CH	1,56 (m)
26	19,21	CH_3	0,84 (d)
27	19,59	CH_3	0,86 (d)
28	23,25	CH_2	1,10 (m)
29	12,17	CH_3	0,83 (d)

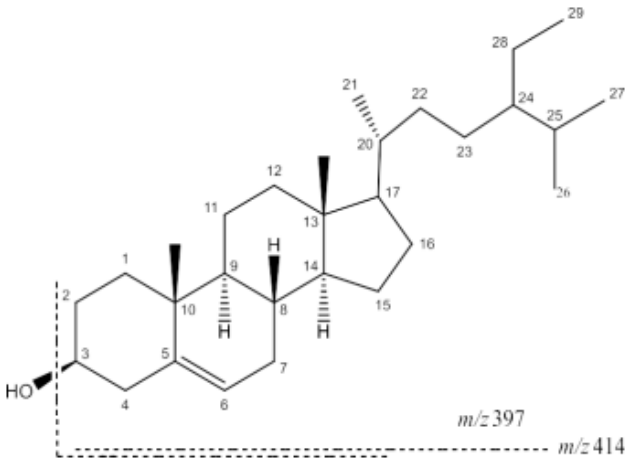


Figure 4. The structure of β-sitosterol

C-methylene, C-methine, or as C-quaternary and to determine the chemical shift of each group the group as the identify of each location of the C atom inside compound. ^{13}C-NMR spectrum shows the presence of 29 atoms carbon isolate. There are 2 (duplicate) alkene carbon atoms located on low magnetic field (downfield), namely at ÿC 121,92 ppm (-CH=) and 140,94 ppm (-C=). Signal complexity at high magnetic field (upfield) can be analyzed using the DEPT (Distortionless Enhancement Polarization by Transfer) technique so that the amounts of -CH_3, -CH_2, -CH-, and C (the quaternary carbon) can be can be determined. DEPT spectral data can be identified with the type of carbon isolate, namely 6 methyl carbon (-CH3); 11 methylene carbon (-CH2); 9 methine carbons (8(-CH) and 1 (-CH=) and 3 quaternary carbon (2(-C-) and 1 (-CH=). Complete results of ^{1}H and ^{13}C-NMR data can be seen Table 2. The chemical structure of the isolate can be seen Figure 4.

CONCLUSION

In this study, *Durio zibethinus* L. was macerated using 96% ethanol solvent, then fractionated with ethyl acetate, water, and n-hexane which was then subjected to phytochemical screening followed by anti-diabetic testing. The isolated steroid compound is in the form of white needle-shaped crystals. The melting point of isolated pure steroid crystals is 132-134^{0}C. The structural characteristics of the isolated steroid compounds using UV-Vis spectrophotometer and infrared spectrophotometer showed that the isolated compounds contained OH groups, methyl groups, and had unconjugated double bonds. The structure of isolated compounds were identified by spectroscopic methods, ^{1}H and ^{13}C-NMR and LCMS.

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