β-SITOSTEROL FROM BARK OF ARTOCARPUS CAMANSI AND ITS ANTIDIABETIC ACTIVITY

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Abstract

Research compound betasitosterol from the bark of plant of Artocarpus camansi (kulu) and test its antidiabetic has been done. The study begins by extracting samples (3.5 kg),w in the solvent of hexane, hexane extracts obtained,then separated by gravity column chromatography, and was characterized by GC-MS. The results obtained are groups of fractions A, and B. From phytochemical test results, the fraction of group B, has a steroid framework. Group fractions B after rechromatographed is obtained a pure isolates of B. Characterization by ^{1}H-NMR and MS (from GC-MS), pure isolate of B is β-sitosterol. Antidiabetic activity test results showed that the extract of n-hexane, fraction group A and fraction group of B (containing β-sitosterol), can lower blood sugar levels male Swiss Webster mice were performed by the method of glucose tolerance. Dose of extract of n-hexane is 50 mg/dL at minute 30th, 60th and 90th can lower glucose as much as 103 mg/dL; 71 mg/dL; and 49.33 mg/dL. Group of fraction A of a dose 50 mg/dL at 60th minutes and 90th can lower blood glucose of mice are: 116 mg/dL; and 58 mg/dL. Group B fraction (containing β-sitosterol) at a dose of 50 mg/dL at minute 30th, 60th and 90th can lower glucose by 73 mg/dL; 87 mg/dL; and 73.33 mg/dL. Group fraction of B is the highest in lowering blood glucose average compared with n-hexane extract and group A. Analysis of variance of this fraction were performed by using ANOVA Post hoc analysis procedures one-way of, significant differences ($p<0.05$) and ($p<0.01$) conducted using Tukey.

Key words: Kulu, Artocarpus camansi, antidiabetic, β-sitosterol.

Introduction

This study is part of research on A. camansi, leaves and the ethyl acetate extract from the bark of this plant has been published previously (Rosnani Nasution at al., 2014, Rosnani Nasution et al., 2015).The same study was also performed by Marianne et al., (2011)

Artocarpus camansi is known as breadnut, this plant in Indonesia is often referred to as kulu and as Artocarpus communis. According to Ragone (2006) A. communis in contrast to A.camansi, but the study of this two plant often do not to distinguish between them (Syah, 2005) states that researchers who conduct research on plant of A. camansi and A. communis has not specified whether the of plants studied are A. camansi or A.communis, thus to distinguish phytochemicals are difficult to do.

Materials and Methods

Material of Plant

Bark of A.camansi plants (kulu) was taken in the Cot Keueung, Darussalam, Banda Aceh in 2014. The plant was identified at Department of Biology, University of North Sumatera, Medanense, Medan.

Spectroscopic Investigation

Mass spectra were measured using a Shimadzu GC-MS QP 2010 Ultra. The ^{1}H-NMR (400 MHz), Spectra were recorded on a JEOL in CD_3Cl. Column chromatography was conducted on silica gel 60 (70-230 mesh, Merck). TLC analysis was carried out by using precoated silica gel plates (Merck).

Testing Phytochemicals

The method used for testing of phytochemical can be found in: Phytochemical methods, Simplified Determination Method to Analyze Plant (Harborne, 1987).

Isolation of Secondary Metabolites From Plant Kulu bark (A. camansi)

Bark of A. camansi already dried as much as 3.5 kg macerated with n-hexane for 2x24 hours and then filtered, then the filtrate was concentrated with a rotary evaporator. Concentrated extract of n-hexane were obtained as much as 155.9 grams (4.45%). After testing, it turns hexane extract was active as hypoglycaemi, and ' the extract was separated by chromatography gravitasi to obtain pure compound or pure isolates were active as hypoglycaemi.

Fractionation Extract Hexane

A total of 30 grams of concentrated n-hexane extract was separated by gravity column chromatography (KKG), with column 3 cm in diameter and with a length of 50 cm. The stationary phase used was silica gel GF 60 254 (70-230 mesh, 150 g, Merck), and using a mobile phase eluent system n-hexane and ethyl acetate in the ratio of 100: 0; 90:10, 80:20;7 0:30; and 60:40. Each fraction was monitored by using plate thin-layer chromatography (TLC). The same stain pattern was combined and obtained two main groups of fractions (A, B). Group B was rechromatographed then washed again with hexane and ethyl acetate solvent and was obtained pure isolates of B. Furthermore, pure isolates were characterized by ^{1}H-NMR. Data from the ^{1}H-NMR is adjusted with MS from GC-MS. To group B fraction (containing isolate B), the group fraction of A, and the hexane extract were conducted testing of antidiabetic.

Antidiabetic Test

Preparation of carboxymethyl cellulose (CMC)
CMC solution of 1% (w/v) was made by dissolving 1 gram of CMC into 100 mL of distilled water, a solution of CMC was given to mice at a dose of 0.5 mL/kg bw.

Glucose Tolerance Test (Frohde and Medeiros, 2008)

Before use, the mice were acclimatized for 7 days in laboratory conditions as well as getting enough food and drinks. After 7 days, selected mice were healthy, characterized by weight stable or increased and did not show any abnormal behavior. Mice were divided into5 groups, each of the groups contain three of mice, group I:diabetic control was given CMC-Na (0.5 %), group II: the standard drug glibenclamide, was given orally at dose of 0,45 mg/kg bw, group III: treated with 50 mg/kg bw (effective dose) of fraction group of B

(contain of isolate B), group IV: treated with 50 mg/kg bw hexane extract of A. camansi, group V: treated with 50 mg/kg bw of fraction group of A. After being given glucose a dose of 3 g/kg bw, all groups of mice were measured in blood glucose levels at 30th minutes, 60th, and 90th minute.

Blood Samples

Mice were put in a box modifications (restrainer), tail cleaned with a wet cotton so that the dirt is gone, then smeared with alcohol 70% v/v. Blood was drawn through the lateral tail vein, which was cut aseptically approximately 1-2 mm from the tip of the tail without anesthesia, blood droplets first removed, then the next drop of blood dripped on the strip One Touch Horizon .

Statistical Analysis

Statistical analysis was performed using Statistical Product And Service Solution (SPSS) Program. Analysis of variance were performed using ANOVA one way Post hoc analysis procedures, significant differences ($p<0.05$) and ($p<0.01$) using Tukey (Santoso, 2012).

Results and Discussion

Phytochemicals test results

Phytochemical yield from fresh bark (A. camansi) contain of alkaloids, steroids, and Terpenoids.

Structure elucidation isolate of B

isolates B is a white solid with a melting point of 135-136^{0}C, spectrum of ^{1}H-NMR isolates shows that the pattern of the spectrum is a derivative of steroid compounds. The characteristics of the steroids spectrum are protons that resonate at region δ 0-2 ppm. Once identified, the pattern spectrum of isolates B is very similar to the pattern spectrum β-sitosterol, so that was compared with compounds β-sitosterol standard (Rosnani at al., 2014). The comparison can be seen in Table 1 below.

Table 1. Comparison of compounds bark A. camansi with β-sitosterol (of propionate) compounds that have been identified

No. of Carbon	Compounds from the stem bark of A. camansi	Compounds from the leaves A. camansi *	No. of Carbon	Compounds from the stem bark of A. camansi	Compounds from the leaves A. camansi *
1	1.01 – 1.06, m	1.01 – 1.06, m	15	1.44 – 1.52, m	1.44 – 1.53, m
	-	1.80 – 1.85, m	16	1.79 – 1. 83, m	1.79 – 1. 83, m
2	1.79 – 1.83, m	1.79 – 1.83, m	17	1.01 – 1. 06, m	1.01 – 1.06, m
	-	1.92 – 2.00, m	18	0.67, s	0.65, s
3	3.47	3.45 – 3.54, m	19	0.97, s	0.98.s
4	1.90 – 2.00,m	1.90 – 2.00, m	20	1.25 – 1.31, m	1.27 – 1.38
5	-	-	21	0.76, d	0.77, d
6	5.11 – 5.12, dd	5.31 – 5.33, d	22	0.96 – 1.01, m	0.95 -1.01, m
7	1.38 – 1.44, m	1.38 – 144, m		1.20 – 1.26, m	1.20 – 1.26, m
	1.79 – 1.83, m	1.79 – 1.83, m	23	1.01 – 1.10, m	1.01 – 1.10, m
8	1.38 – 1.44, m	1.38 – 1.44, m	24	0.84-0.88, m	0.83 – 0.88, m
9	0.86 – 0.93, m	0.86 – 0.91, m	25	1.60 – 1.65, m	1.61 – 1.66, m
10	-	-	26	0.84, d	0.8148, d
11	1.41 – 1.45, m	1.40 – 1.53, m	27	0.79, d	0.7965,d
12	2.21 – 2.23, m	2.21 – 2.26, m	28	1.20 – 1.31, m	1.20 – 1.28, m
13	-	-	29	0.88, d	0.88, d
14	0.93 – 1.01, m	0.90 – 1.01, m			

*(Rosnani Nasution, at al., 2014)

Resonance proton Singlet appears in the methyl group H-18 (δ 0.67 ppm) and H-19 (δ 0.97 ppm). For other methyl groups, present in the proton H-21 (δ 0.76 ppm), H-26 (δ 0.84 ppm), H-27 (δ 0.79 ppm) and H-29 (δ 0.88 ppm) that resonates as a doublet doublet. Proton H-3 (δ 3.47 ppm) binding hydroxyl groups. Resonance at low magnetic field seen in the proton H-6 (δ 5.29 to 5.34 ppm), which resonates as a doublet-doublet, characteristic of protons in the atom C-6. For other protons resonate between δ 0.84 to 2.23 ppm as a multiplet that are characteristic of CH and CH_2. The suggestion isolate of B is a ß-sitosterol was reinforced by spectral data of its MS (from GC-MS). The MS data of isolate B can be seen in Figure 1.

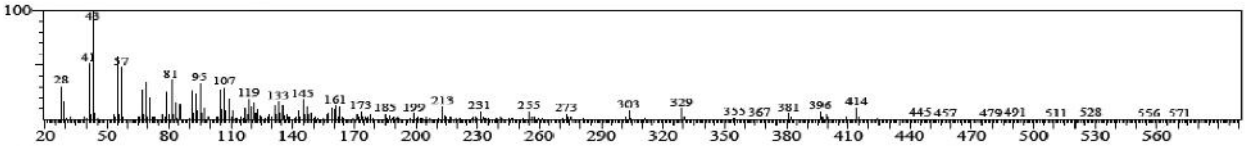

Figure 1. The mass spectrum of β-sitosterol

The spectrum of Figure 1, is a very typical spectrum for derivative of sitosterol group, the peak ion m/z 396, is thought to be derived from the loss of molecules of wáter (H_2O) from molecules of β-sitosterol (m/z 414). Ion peak at m/z 396 was later releases a methyl group ($-CH_3$) and produces a peak ion m/z 381. Termination of the side chain of m/z 414 ion produce a peak at m/z 273,the next release of water molecule to produce peak ion m/z 255.

Based on the fragmentation pattern in Figure 1, the proposed compound from fraction group of B is a compound β-sitosterol which has 29 carbon atoms and having one double bond in its steroid framework. The structure of these compounds as in Figure 2.

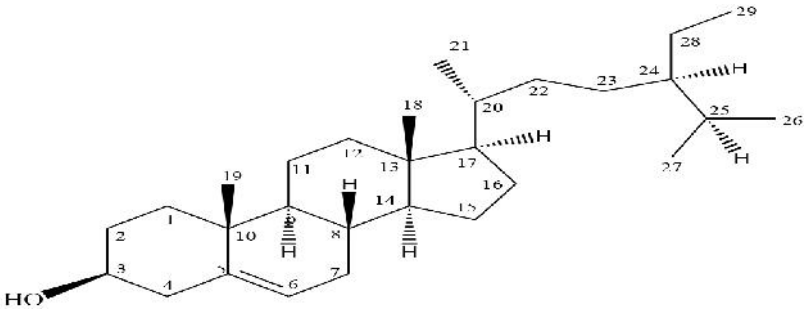

Figure 2. The compound β-sitosterol

Results Test Blood Glucose Mice After administration of n-hexane extract, Groups fractions of A and B from bark Kulu.

The results of measurements of the average blood glucose levels in mice can be seen in Figure 3.

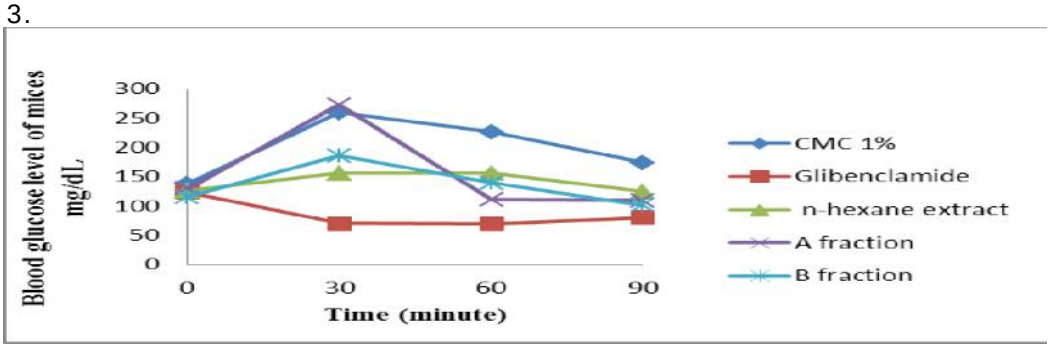

Figure 3. Curve average decrease in blood glucose levels of mice after administration of n-hexane extract, fraction group A and fraction group B of stem bark of A. camansi

Based on Figure 3 can be seen the average initial blood glucose in mice in a state of uniform, looks average initial blood glucose levels are in the range of 115.33 to 137.67 mg/dL. At 30th minutes after administration of glucose, n-hexane extract can lower blood glucose of mice (156 mg/dL) lower than the negative control (259 mg/dL), the fraction of group A cause a rise in blood glucose of mice (273 mg/dL) , the increase is relatively higher than the negative control mice blood glucose which increase blood glucose only at 259 mg/dL. Fraction B lowers blood glucose of mice (186 mg/dL) lower than the negative control. Blood Glucose of mice given glibenclamide (positive control) was 70 mg/dL, indicating measurement process according to the procedure. At minute 60th, extract n-hexane, group fractions A and B groups can lower blood glucose of mice is lower than blood glucose of the negative control mice. Blood glucose of mice given n-hexane extract (155 mg/dL), was given with group fractio A (110.33 mg/dL), and given the fraction group B (139.33 mg/dL) were lower than blood glucose negative control of mice (226.33 mg/dL), whereas mice was given with glibenclamide the blood glucose was 69.67 mg/dL. In the 90th minute, n-hexane extract, group fractions A and B groups can lower blood glucose of mice is lower than the blood glucose negative control mice. Blood glucose of mice was given n-hexane extract (174 mg/dL), was given group fraction A (109.33 mg/dL), and given the fraction group B (100.67 mg/dL) lower than blood glucose negative control mice (174 mg/dL), whereas mice given blood glucose glibenclamide was 80 mg/dL.

Furthermore, blood glucose negative control mice was reduced with each blood glucose of mice given the extract of n-hexane, fraction groups A and B at the same minute. The result of this reduction can be seen in Figure 4.

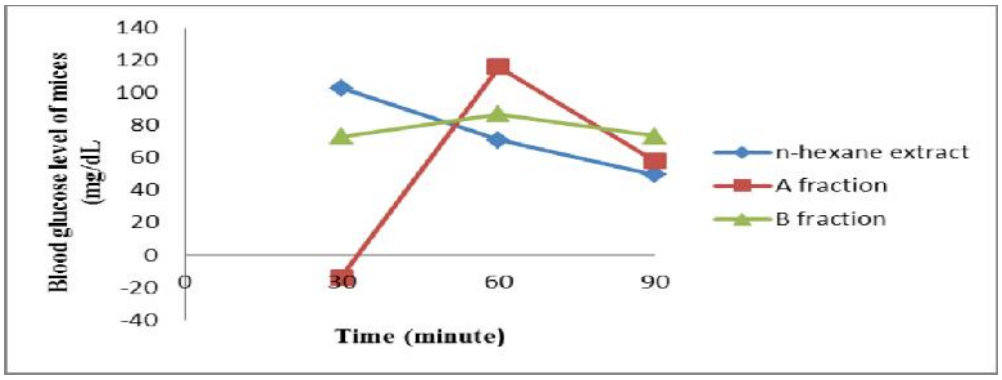

Figure 4. Difference in average reduction in blood glucose levels of negative control mice with blood glucose of mice were given the extract of n-hexane and fraction groups A and B

From Figure 4 it can be seen that the n-hexane extract of the minutes to 30th can lower blood glucose of mice 103 mg/dL, at 60th minutes can lower blood glucose by 71 mg/dL and at 90th minutes can lower blood glucose of mice 49.33 mg/dL.

Giving group fraction A to mice at 30th minutes even raise the blood glucose levels of mice by -14 mg/dL, but in the 60th minute fraction A can lower blood glucose sharply mice in the amount of 116 mg/dL. Later in the 90th minute, group fraction of A can lower blood glucose by 58 mg/dL.

The increase in blood sugar levels of mice could happen, because compound A, has not yet worked, so it has hyperglycaemia effect (Jagtap at al., 2010). Giving fraction B (containing β-sitosterol) in mice, at minute 30th, can lower blood glucose by 73.33 mg/dL. At 60th minutes can lower blood glucose of mice at 87 mg/dL, and in the 90th minute, fraction B can lower blood glucose of mice up to 73.33 mg/dL. Overall the n-hexane extract, fractions A and B groups can lower blood glucose when compared to the negative control were only given 1% CMC. Fraction B can lower blood glucose better than the n-hexane extract and fractions group A.

Compounds β-Sitosterol As Antidiabetic

Searching of the literature states that the compounds β-sitosterol has a diverse variety of biological activities, among others, as antidiabetic, drug of cardiovascular disease, cancer prevention, anti-inflammatory, hypocholesterolemic activity, antioxidants and others (Saedina at al., 2014). High glucose levels in the blood tend to increase cholesterol levels. This is due to insulin deficiency affects the metabolism of carbohydrates, lipids and proteins. Insulin influential on lipid metabolism and can increase the synthesis of fatty acids in adipose tissue (Suryawansh at al., 2006).

From the research that has been done n-hexane extract and fractions A and B groups (containing β-sitosterol) from Artocarpus camansi bark can lower blood sugar levels of male Swiss Webster mice.

Conclusions

From the research that has been done can be concluded that:
Extract n-hexane, the fraction of group A and group fraction B able to lower blood glucose levels were tested in male Swiss Webster mice. Extract the n-hexane at minute 30th, 60th and 90th can lower blood glucose are 103 mg/dL; 71 mg/dL; and 49.33 mg/dL. A group of fractions can decrease glucose at minute 30th, 60th and 90th at -14 mg/dL; 116 mg/dL; and 58 mg/dL, and group fractions B in minute of 30th, 60th and 90th can lower blood glucose by 73 mg/dL; 87 mg/dL; and 73.33 mg/dL. Fraction B showed greater activity lowering blood glucose compared with extract n-hexane and fractions group A. Based on the ^{1}H-NMR and MS spectra the compounds suspected is β-sitosterol

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