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Isolation of Chemical Compounds from Root of *Artocarpus camansi* and Antidiabetic Activity Test

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ABSTRACT

The antidiabetic test of crude extract of n-hexane, group of fraction A, group of fraction B, group of fraction C, group of fraction D and isolate of C43 from root skin of Kulu (A. camansi), it is known that group of fraction A can lower blood sugar of mice is the biggest, that is at min 60th, followed by crude extract of n-hexane, group of fraction B, group of fraction C, group of fraction D and isolate C43. The C4 3 isolate the largest of which decreased blood glucose in the 30th min, but in the 90th min raised blood glucose in mice. The fraction group of A had lower mean blood glucose levels and significantly different from the positive control with 95% confidence level ($P<0.05$) at min 120th. The result of the separation of fraction group guided by activity anti-diabetes obtained isolate C43, The isolate of C43 after they were characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR and MS (GC-MS), were suggested as lupeol acetate.

Keywords: Kulu (*Artocarpus camansi*), Lupeol acetate, Antidiabetes

INTRODUCTION

Diabetes mellitus is one of the five major causes of death in the world. Indonesia is the fourth most populous country in the world in 2010 with a population of 237.6 million people and has the seventh largest number of patients (7.6 million) [1]. Treatment of diabetes mellitus can be done using Oral Hypoglycemic Drugs (OHO), but with high cost and high side effects due to long treatment time, so the alternative treatment of Diabetes mellitus today often re-utilize traditional medicine derived from plants [2], among them the plant *A. camansi*, belonging to the family Moraceae. Ethanol extract of *A. camansi* leaf has the ability to decrease blood glucose level in mice with dose 50 mg/kg BW [3]. Research on leaves of *A. camansi* produces steroid compounds, i.e., β-sitosterol propionate from n-hexane extract, which is active as an antidiabetic [4]. *A. camansi* plant stem bark produces triterpenoid compounds, namely β-amyrin acetate [5], whereas the research at the skin of the roots is still relatively small. Based on the above, it is necessary to study the skin of the root of *A. camansi*, to obtain information of secondary metabolite compounds contained in the root skin extract of the plant, the biological activity of its active compound as an antidiabetic drug, and the structure of the compound which is active as antidiabetic.

MATERIALS AND METHODS

Plant materials

The sample used in this research is root skin of *A. camansi* plants collected in Lam me District of Kuta Baro, Banda Aceh. Bioindicators used in this study is a male Swiss Webster mice.

Testing phytochemicals

The method used for testing of phytochemical can be found in phytochemical methods, simplified determination method to analyze plant [6].

Spectroscopic investigation

Mass spectra were measured using a Shimadzu GC-MS QP 2010 Ultra. The 1D, Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectrum was measured in a $CDCl_3$ solvent with a 500 MHz JEOL spectrophotometer Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) measured in a Deuterated Chloroform ($CDCl_3$) solvent with a 125 MHz JEOL Spectrophotometer. Column chromatography was performed on silica gel 60 (70-230 mesh Merck). Thin Layer Chromatography (TLC) analysis was carried out by using precoated silica gel plates (Merck).

Extraction of *A. camansi* Root

A. camansi root skin sample was 3.1 kg which has been dried, then macerated with n-hexane solvent for 3 × 24 h, then filtered. The obtained filtrate was then evaporated with a rotary evaporator to obtain 139.15 g (4.44%) of concentrated n-hexane. N-hexane extracts were tested for phytochemicals and their antidiabetic activity, and characterized by Gas Chromatography-Mass Spectrometry (GC-MS).

Fractionation n-hexane extract

The 30.01 g of concentrated n-hexane extract was fractionated using gravitational column chromatography. The eluent system used is n-hexane elution gradient system: ethyl acetate with the ratio of n-hexane (100%), n-Hexane: Ethyl acetate (90:10); n-Hexane: Ethyl acetate (80:20); n-Hexane:Ethyl acetate (70:30) and n-Hexane:Ethyl acetate (50:50). The separation result was 77 fractions after being monitored by TLC. The same stain pattern is combined so that 4 fraction groups are obtained. Fraction A, of 3.23 g, is yellow, contains terpenoids, fraction B, as much as 20.63 g, colored orange, containing steroids. Fraction C, as much as 4.22 g, brown, containing terpenoids; And fraction D, as much as 1.49 g, brown containing terpenoids/steroids. After antidiabetic testing, it was known that the fraction group C was active, so chromatography was repeated to group C.

The fraction group C of 2.67 g was re-chromatographed with a column of gravity chromatography to obtain the pure compound with the n-hexane eluent system:Ethyl acetate (95:5). The relatively pure 12 (C4) fraction was found to be 0.26 g. The fraction group C4 was re-chromatographed with the gravity column with the system of eluent, n-Hexane:Ethyl acetate (97:3) and obtained a relatively pure, fraction 7-20 (C43) fraction tested antidiabetic activity against Swiss Webster mice, then characterized by FTIR, ^{1}H-NMR and ^{13}C-NMR.

Glucose tolerance test [7]

Testing of antidiabetic activity was done by using glucose tolerance method. Prior to treatment, the mice first acclimatized for 7 days and on the last day the mice are fasted for 20-24 h and measured blood glucose levels and weight. Mice then divided into 8 groups, each group consists of 3 mice. The negative control group was given CMC 1% with a dose of 1 mg/kg BW. A positive control group was given glibenclamide at a dose of 0.45 mg/kg BW. The sample was suspended in CMC, administered to mice at a dose of 50 mg/kg BW [3]. After 30 min, all treatment groups were given glucose with 50% concentration of 3 g/kg BW orally. Measurement of blood glucose levels of mice was measured every 30, 60, 90 and 120 min after glucose administration.

Samples blood

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 from 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 was performed using ANOVA one way and ($P<0.05$) using Tukey [8].

RESULTS AND DISCUSSION

Phytochemical test results

Phytochemical results of fresh samples and extract n-hexane root skin of *A. camansi* showed the presence of secondary metabolites of the steroid and terpenoids groups. Group of fraction A, fraction group of C, and $C4_3$ isolate., these samples containing terpenoids, while the fraction group B contains steroids, and the D fraction group contains terpenoids and steroids.

Characterization of isolate $C4_3$

Isolate $C4_3$ is white solid, analysis of ^{1}H-NMR spectra and ^{13}C-NMR of isolate $C4_3$ as follows. The ^{1}H-NMR spectrum shows the presence of eight methyl of singlets in a chemical shift (δH, ppm), is 0.74, 0.84; 0.86, 0.88, 0.93, 1.06, 1.68 and 2.28 indicating protons (H-28, H-24, H-25, H-23, H-28, H-26, H-30 and H-2 '). Two protons appeared at δH=4.66 ppm and 4.71 ppm as the singlet, showing protons on H-29a and H-29b. Atom H in C-methine (C-3) shows a chemical shift of 4.47. This is because the protons attached to the C-methine (C-3) atom are affected by the electronegativity of the O atoms of the ester group at C-3, causing a large chemical shift value. The result of characterization of isolate $C4_3$ with the ^{1}H-NMR instrument in Figure 1 below:

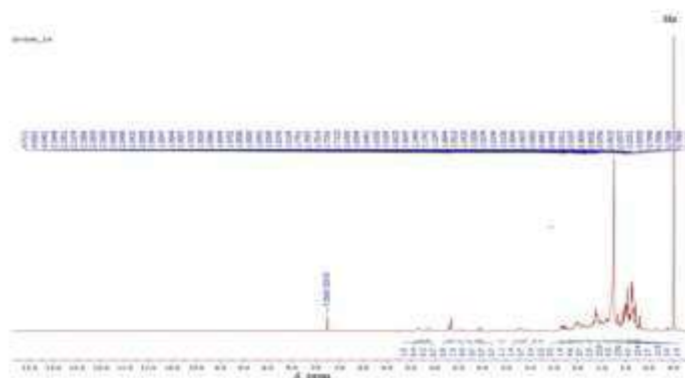

Figure 1: ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) of $C4_3$ isolates

The ^{13}C-NMR spectrum analysis, showing the C-1' carbonyl group at δC=157.04 ppm, C-3 at δC=80.77 ppm and C-20 alkene at δC=150.34 ppm and C-29 at δC=109.51 ppm. The result of characterization of isolate $C4_3$ with the ^{13}C-NMR instrument can be seen in Figure 2 below.

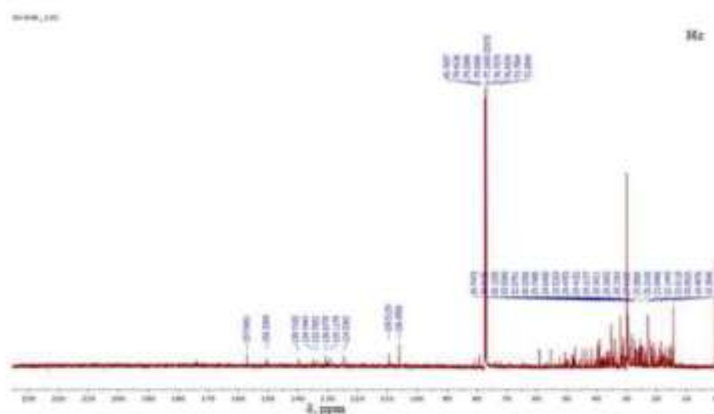

Figure 2: ^{13}C-NMR spectra ($CDCl_3$, 125 MHz) isolate $C4_3$

Spectra ^{1}H-NMR and ^{13}C-NMR of isolates of $C4_3$ are very similar to the spectrum of ^{1}H-NMR and ^{13}C-NMR of lupeol acetate so the isolates $C4_3$ is compared to standard-lupeol acetate to see the equation [9]. Comparison of ^{1}H-NMR and ^{13}C-NMR spectra data of isolates $C4_3$ with lupeol acetate standard are listed in Table 1.

Table 1: Comparison of ^{1}H-NMR and ^{13}C-NMR spectra, isolates of $C4_3$ with lupeol acetate compounds (^{1}H-NMR, $CDCl_3$, 500 MHz and ^{13}C-NMR, $CDCl_3$, 125 MHz)

Position	Isolate $C4_3$		Lupeol acetate Standard [9]		Position	Isolate $C4_3$		Lupeol acetate Standard [9]	
	δ_H (ppm)	δ_X (ppm)	δ_H (ppm)	δ_X (ppm)		δ_H, (ppm)	δ_X (ppm)	δ_H (ppm)	δ_X (ppm)
1	1.51 (m)	38.57	1.51 (m)	38.1	18	1.56 (m)	49.01	1.56(m)	48
	1.73 (m)		1.72 (m)						
2	1.52 (m)	21.71	1.50 (m)	27.9	19	2.36 (m)	48.14	2.38(m)	48.3
	1.71 (m)		1.70 (m)						
3	4.47 (dd)	80.77	4.48 (dd)	80.6	20	-	150.34	-	150.9
4	-	37.79	-	38.1	21	0.88 (m)	30.06	0.88(m)	29.4
						1.06 (m)		1.08(m)	
5	0.70 (m)	55.37	0,70 (m)	55.4	22	1.35 (m)	40.15	1.33(m)	40
						1.48 (m)		1.46(m)	
6	1.43 (m)	18.37	1.44 (m)	18.1	23	0.88 (s)	27.42	0.88 (s)	28
	1.53 (m)		1.53 (m)						
7	1.25 (m)	34.57	1.25 (m)	31.9	24	0.84 (s)	16.76	0.84 (s)	16.6
	1.49 (m)		1.49 (m)						
8	-	40.61	-	40.9	25	0.86 (s)	16.37	0.84 (s)	16.2
9	1.30 (m)	50.52	1.30 (m)	50.4	26	1.06 (s)	16.35	1.03 (s)	16
10	-	37.28	-	37.1	27	0.93 (s)	14.87	0,94 (s)	14.1
11	1.25 (m)	20.85	1,25 (m)	21	28	0.74 (s)	18.19	0,76 (s)	18
	1.48 (m)		1.47 (m)						
12	1.51 (m)	23.84	1.51 (m)	25.1	29a	4.66 (s)	109.51	4,57(m)	109.4
	1.61 (m)		1.61 (m)						
13	1.63 (m)	36.27	1.63 (m)	37.8	29b	4.71 (s)	-	4,71 (d)	-
14	-	43.28	-	42.8	30	1.68 (s)	19.46	1.68 (s)	19.3
15	1.23 (m)	25.32	1.13 (m)	25.2	1’		157.04		179.7
	1.38 (m)		1.41 (m)						
16	1.42 (m)	35.71	1.42 (m)	34.9	2’	2.28 (S)	28.23	2.28 (s)	34.9
	1.55 (m)		1.56 (m)						
17		43.45		43.2					

Based on Table 1 above, it shows that the C4$_3$ isolate corresponds to the chemical shift value (δH) of the lupeol acetate standard. In addition, the lupeol acetate compound is also present in the plant *Artocarpus nitidus* [10], and is also present in the leaves of the *Ficus racemosa* plant [11]. This situation is probably closely related to *A. camansi*, because the plants *A. nitidus* and *F. racemosa* plants are the plants of a family of Moraceae. Thus, based on the chemotaxonomic approach of isolate structure C4$_3$ is suggested as lupeol acetate.

Isolate C4$_3$ as lupeol acetate is reinforced by MS data. The fragmentation patterns of C4$_3$ isolates occurring on m/z, 453, 408, 357, 218, 189, 109, 43 (100%) are characteristic of lupeol acetate [12,13], there is loss of methyl group on molecular ion m/e 468. Frame fragmentation of C4$_3$ isolate can be seen in Figure 3 below.

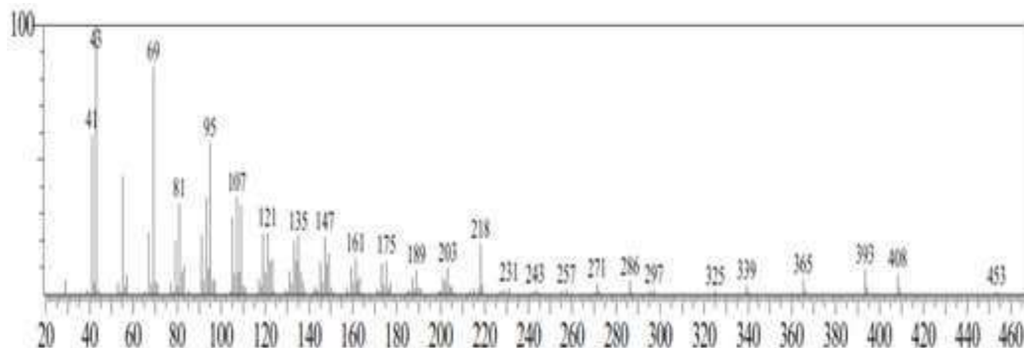

Figure 3: Spectrum of MS isolates C4$_3$

The C4$_3$ isolate as lupeol acetate was also strengthened by data from the FT-IR spectrum showing the presence of a carbonyl group (C=O) ester at the wave number 1735.09 cm^{-1}. Based on the results of the characterization of ^{1}H-NMR and ^{13}C-NMR, MS and FT-IR suspected isolates C4$_3$ is lupeol acetate. The structure of lupeol acetate can be seen in Figure 4.

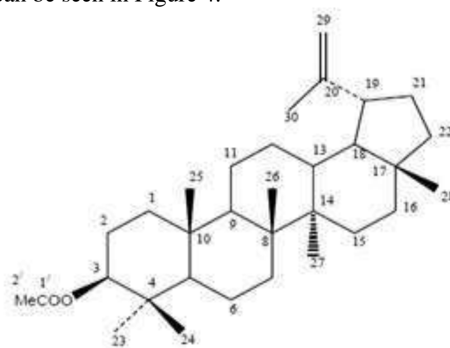

Figure 4: Structure of lupeol acetate compounds [14]

Antidiabetic activity

The antidiabetic activity of n-hexane *A. camansi* extract, fraction group A, B, C, D, C4$_3$ isolate, CMC control and positive control (glibenclamide) in reducing blood glucose levels of mice can be seen in Figure 5.

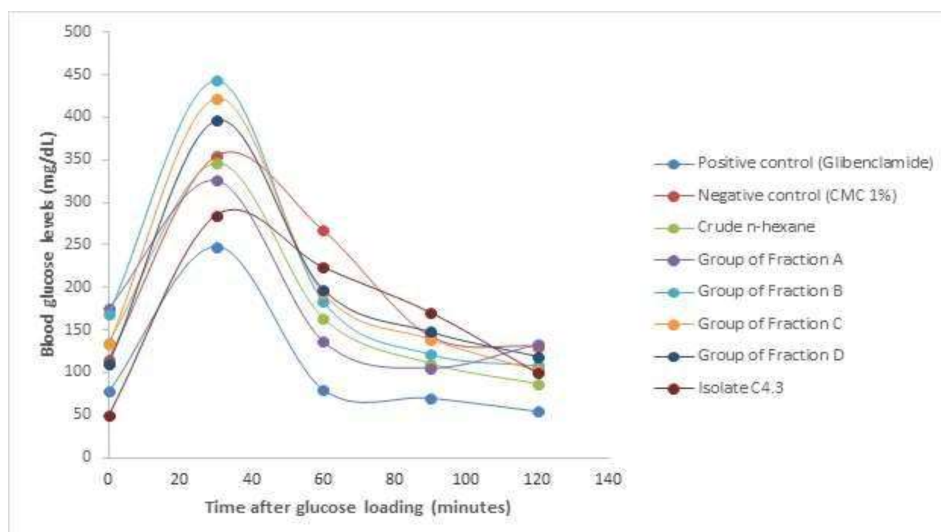

Figure 5: Graph of decreased/increased blood glucose levels of mice

Based on Figure 5 it is known that at 30 min after being given glucose resulted in blood glucose level in all mice given crude extract, group of fraction A, group of fraction B, group of fraction C, group of fraction D, isolate $C4_3$, positive control and negative control had increased blood glucose levels. This is because all samples have not worked to control glucose in the blood.

Blood glucose levels of mice given n-hexane crude extract at 30 min increased blood glucose to 346 mg/dl. However, in the 60th min, the 90th and the 120th decreased the respective blood glucose to 164, 110.67 and 87 mg/dl. Mice given the fraction group of A, at min 30, have increased blood glucose become 325.67 mg/dl. In the min 60th decreased blood glucose to 133.00 mg/dl and min 90th and 120 min to 136.67 mg/dl and 105.33 mg/dl, respectively. Mice given the fraction group B experienced an increase in blood glucose at min 30th, i.e., 442.67 mg/dl and decreased blood glucose at min 60th, 90th and 120th, respectively, 183.67, 121.33 and 107.67 mg/dl. The mice given the C fraction group at 30th min experienced an increase in blood glucose to 422 mg/dl and decreased blood glucose levels at min 60th, 90th, 120th is 193.00, 139.00 and 102.33 mg/dl respectively.

Mice given the fraction D group had elevated blood glucose at 30 min to 395.67 mg/dl and then blood glucose decreased at men to 60th, 90th, and 120th min respectively 197.67, 147.67 and 119.00 mg/dl. Mice given $C4_3$ isolate at min 30, an increase of blood glucose level of the mouse to 284.67 mg/dl. At 60th, 90th and 120th min decreased blood glucose levels to 223.67 mg/dl, 170.67 mg/dl and 99.33 mg/dl. Mice were given glibenclamide positive control at 30th min after glucose administration showed an elevated blood glucose level to 248.00 mg/dl, at 60th, 90th and 120th min and decreased blood glucose to 80, 70 and 54.33 mg/dl. Mice were given a negative control at the 30th min after glucose administration showed an elevated blood glucose level to 353.33 mg/dl and decreased blood glucose levels at 60th, 90th, 120th, to 267.00, 142.67 mg/dl and 130.67 mg/dl.

Furthermore, the activity of lowering the blood glucose in the mice was calculated by reducing the blood glucose of the negative control mice, with the mice blood glucose administered: crude extract n-hexane, fraction A group, fraction group B, C faction group, D fraction group and $C4_3$ isolate. The result of the reduction of blood glucose level of the negative control group with six test preparation in Figure 6.

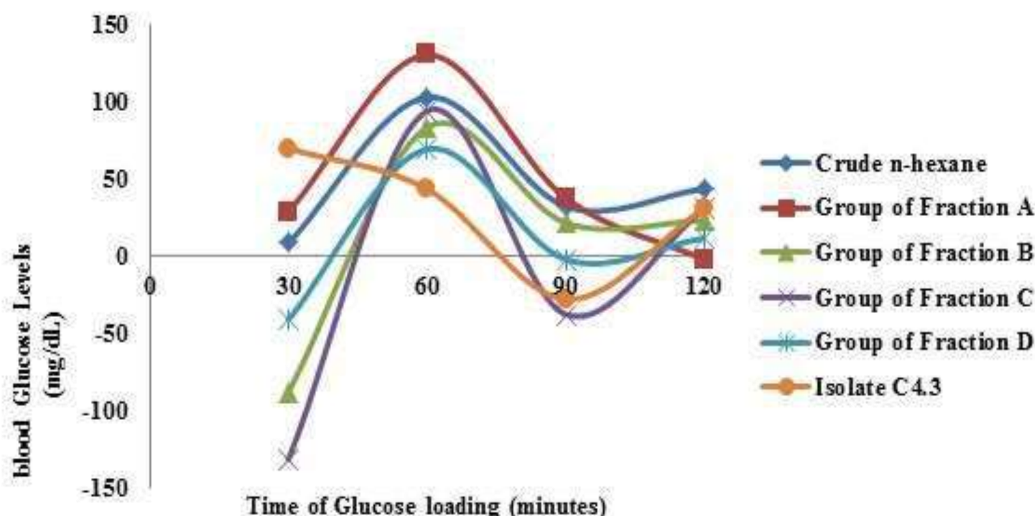

Figure 6: Yield of reducing blood glucose of control negative of mouse (CMC) with blood glucose level of mice given crude extract, fraction group A, group of fraction B, group of fraction C, group of fraction D and isolate of $C4_3$

Based on the subtracting of blood glucose level between control/CMC with n-hexane extract, group of fraction A, group of fraction B, group of fraction C, group of fraction D, and isolate $C4_3$ as followed. At min 30th, $C4_3$ isolate can decrease blood glucose of mice by 69.67 mg/dl, group fraction of A can lower blood glucose of mice by 28.67 mg/dl and crude extract of n-hexane decrease blood glucose mice by 8.33 mg/dl. Groups of fraction B, the group of fraction C and group of fraction D increased blood glucose of each mice by 88.33, 67.67 and 41.33 mg/dl. In the 60th min, the fraction group A lowered the blood glucose of mice (130.33 mg/dl), the crude extract of n-hexane lowered the blood glucose of mice by 103.00 mg/dl, the B group fraction decreased the blood glucose of mice (83.33 mg/dl), the C fraction group decreased the mice blood glucose (74 mg/dl), the fraction D group decreased the mice blood glucose (69.33 mg/dl), and the $C4_3$ isolate decreased the mice blood glucose (43. 33 mg/dl). In the 90th min, the fraction group A lowered the mice blood glucose (37.33 mg/dl), the crude extract of n-hexane lowered the mice blood glucose (32.00 mg/dl), the B group fraction decreased the mice blood glucose (21.33 mg/dl), and the C fraction group lowered the blood glucose of mice by (3.67 mg/dl). Administration of fraction group D and isolate of $C4_3$, increased the blood glucose of each mouse 2.33 mg/dl and 28.00 mg/dl against the negative control. At min 120th, the fraction groups A increased blood glucose by 2.33 mg/dl. Isolates $C4_3$ decreased mice blood glucose by 31.33 mg/dl. Groups of fraction A, group of fraction C and group of fraction D each decreased blood glucose of mice (43.67 mg/dl), (28.33 mg/dl), D (1.67 mg/dl).

Based on the above, the highest decrease of blood glucose was the group of fraction A, that is at min 60th, then crude extract of n-hexane, group of fraction B, group of fraction C, group of fraction D and isolate $C4_3$. The $C4_3$ isolate group, the largest of which decreased blood glucose in the 30th min, but in the 90th min raised blood glucose in mice. To see the difference in blood glucose reduction activity in Swiss Webster male mice between samples and controls using SPSS program, the analysis used ANOVA one-way Post hoc analysis using Tukey, to obtain the following Table 2.

The fraction group A having an average blood glucose level was lower than that of the control group and significantly different to only glibenclamide ($P<0.05$) occurring at 120th min.

Table 2: Comparison of blood glucose decrease between crude extract n-hexane, fraction group of A, fraction group B, fraction group C, fraction group of D and isolate $C4_3$, p value (<0.05) against glibenclamide (positive control) and CMC 1% (negative control)

Groups	Blood glucose level (mg/dl)							
	Min 30th	P	Min 60th	P	Min 90th	p	Min 120th	P
Glibenclamide	248.00	-	80.00	-	70.00	-	54.33	-
		-		-		-		-
CMC	354.33	106.33	267.00	187.00	142.67	72.67	130.67	76.33
		-		-		-		-
crude extract n-hexane	346.00	98.00	164.00	84.00	110.67	40.67	87.00	32.67
		-8.33		-103.00		-32.00		-43.67
Fraction Group of A	325.67	77.67	136.67	70.00	105.33	35.33	133.00	78.67*
		-28.67		-103.00		-37.33		2.33
Fraction Group of B	442.67	194.67	183.67	103.67	121.33	51.33	107.67	53.33
		88.33		-83.33		-21.33		-23.00
Fraction Group of C	422.00	174.00	193.00	113.00	139.00	69.00	102.33	13.00
		67.67		-74.00		-3.67		-63.00
Fraction Group of D	395.00	36.67	197.67	-117.67	147.67	77.67	119.00	64.67
		69.67		-69.33		5.00		-11.67
Isolate $C4_3$	284.67	-36.67	223.67	143.67	170.67	100.67	99.33	45.00
		-106.33		-43.33		28.00		-31.33

Description: Mean value of blood glucose level; (*)=Different meaning to the control; P=Group significance test number, first row of positive control (glibenclamide), second row of control (CMC)

CONCLUSION

All of the samples, crude extract n-hexane, fraction group A, fraction B group, Fraction C group, Fraction D group and $C4_3$ isolate had blood glucose lowering activity in mice, which was best is in the 60th min. The best group of fraction lowering blood glucose of mice was fraction group A, by 130.33 mg/dl, at min 60th, followed by crude extract of n-hexane, can lower blood glucose of mice as much as 103,00 mg/dl, The fraction B group lowered the blood glucose of mice (83.33 mg/dl), and the other group. The fraction group A can lower an average blood glucose level smaller than that of the control group and was significantly different from the control glibenclamide at 95% ($P<0.05$) occurring at 120th min. The $C4_3$ isolate group, the largest of which decreased blood glucose in the 30th min by 69.67 mg/dl, but in the 90th min raised blood glucose in mice. The result of characterization of isolate $C4_3$ using instrument ^{1}H-NMR, ^{13}C-NMR, FT-IR, MS (GC-MS), so the $C4_3$ is thought to be lupeol acetate compound.

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