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Antidiabetic Activity of Murbei (*Morus alba*) from Aceh

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ABSTRACT

Leaves of Morus alba as much as 3 kg was macerated, with n-hexane, and produce hexane extracts as much as 59.51 g (1.98%). The hexane extract as much as 35 g, separated by gravity column and obtained four groups of fractions, namely the fraction A, B, C and D. Group fraction A, that is quite clean re chromatographed to obtain compounds A1. The compound of A1, then characterized by Proton Nuclear Magnetic Resonance (^{1}H-NMR), Carbon-13 Proton Nuclear Magnetic Resonance (^{13}C-NMR), Fourier-Transform Infrared Spectroscopy (FTIR) and Mass spectrometry, and A1 isolates is believed to Ar-turmerone. Antidiabetic test performed by the method of glucose tolerance. n-hexane extract, fraction group A, fraction group B, fraction group C, fractions group D and compound A1, respectively showed a decrease in blood glucose levels in mice at min 30th in the amount of 155.67, 147.00, 140, 226.33, 196 and 200.67 mg/dl. At min 60th, the six of samples lowering blood glucose levels in mice as much as 167, 175.33, 161.67, 162.67, 166 and 180 mg/dl. In the 90th min, the 6 of samples capable of lowering blood glucose levels in mice by 68, 66.33, 45.67, 62, 65.33 and 56.33 mg/dl. At min 120th, the six of samples capable of lowering blood glucose levels in mice as much as 50.67, 65, 36.67, 43.33 57.33 and 67.33 mg/dl. Isolates A1 and group fraction D was the most active lowers blood glucose Swiss Webster of mice, as compared to blood glucose of negative control of mice. All samples are not significantly different from the positive control, glibenclamide, (P<0.05), which shows all the samples are relatively active as the lowering of blood sugar.

Keywords: *Morus alba*, Tolerance glucose, Antidiabetic, Ar-turmerone

INTRODUCTION

World Health Organization (WHO) states that diabetes mellitus is a disease that ranks 9th leading cause of death worldwide [1]. Diabetes mellitus is a chronic disease that is caused due to an abnormal increase in postprandial blood glucose levels [2]. One plant that has been used traditionally as antidiabetic drugs is *Morus alba* (mulberry) [3], which is one fruit crop is classified in this type of mulberry. Based on the study of literature, the leaves of *M. alba* have antiatherosclerosis activity, antihypertensive, antiobesity, antidiabetes, liver proteins, antiviral and antimicrobial [4]. The leaves of mulberry contain triterpenes (lupeol), sterols (β-sitosterol), bioflavonoids (Rutin, moracetin, quercetin-3-triglucoside and isoquercitrin), coumarin and volatile oil [5]. The methanol extract of the leaves of *M. alba* can lose weight bioindicators (rat) to 18.88% [6] and can lower blood sugar by 31.8% in rabbits [7]. The extracts n-hexane and ethyl acetate mulberry leaves originating from Aceh contain terpenoids and steroids; can lose weight mice on average 4-5% [8].

MATERIAL AND METHODS

Plant material

The sample used in this study is *M. alba* leaves collected from Miruk village, Krueng Barona Jaya sub district, Aceh Besar. Bio-indicators used in this study is mice male Swiss Webster.

Spectroscopic investigation

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

Testing phytochemicals

The method used for testing of phytochemicals can be found in phytochemical methods, simplified determination method to analyze plant [9].

Extraction and isolation of terpenoids (Ar-turmerone) from a leaf of *M. alba*

M. alba leaf that has been refined and dried as many as 3 kg dried and macerated for 2 × 24 h, using n-hexane and after evaporated with a rotary evaporator, obtained, extract n-hexane as much as 59.51 g (1.98%). Then, extract of n-hexane was tested phytochemical, antidiabetic activity, and characterized using Gas Chromatography-Mass Spectrometry (GC-MS).

Fractionation of hexane extract

N-hexane extracts *M. alba* of leaves as much as 35 g, was fractionated by column chromatography gravity. Separation is done by elution gradient (increased polarity) using the eluent system (n-hexane: Ethyl acetate) ratio (100:0, 90:10, 85:15, 70:30). Separation results obtained 90 fractions; based stain patterns fractions obtained 4 groups: those fractions A (6-12), a total of 1.93 g, yellow, are positive for triterpenoids. Fraction B group (13-38), a total of 3.16 g, brownish, positive for triterpenoids. Fraction C groups (39-44), a total of 1.28 g, solid green, are positive for steroids. Fraction group D (51-90), a total of 3.59 g, is solid green, are positive for a steroid. The group fraction of A are cleaner than those fractions B, C and D, which were re-chromatographed again, and obtained isolates A1. All group fractions of A, B, C, D and isolates A1 are tested for phytochemical and hypoglycemia activity.

Glucose tolerance test [10]

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, stable weight or characterized by increased and did not show any abnormal behavior. Mice were divided into 8 groups, each of the three groups of mice contain. Group I: Diabetic control was given Carboxy Methyl Cellulose Sodium (CMC-Na) 1%, group II: The standard drug glibenclamide was given orally at a dose of 0.45 mg/kg BW, group III: Treated with 50 mg/kg BW (effective dose), [11], of fraction group of A (contain A1 isolates), group IV: Treated with 50 mg/kg BW, hexane extract of *M. alba*. Group V: Treated with 50 mg/kg BW, of a fraction group of B. Group VI: Treated with 50 mg/kg BW of fraction group of C, Group VII: Treated with 50 mg/kg BW of fraction group of D, Group VIII: Treated with 50 mg/kg BW, isolates A1. The extract and the fraction group A, B, C, D and pure isolates were suspended with 1% CMC-Na.

Having fasted for 20-24 h, the weight of mice were weighed, fasting blood glucose levels were measured and given treatment (above). After 30 min later, the entire group was given a dose of 3 g glucose/kg BW orally. Furthermore fasting blood glucose levels were recorded at 30, 60, 90, and 120 min after glucose loading.

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 from the lateral tail vein, which was cut aseptically Approximately 1-2 mm from the tip of the tail without anesthesia, blood droplets removed first, 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 one-way ANOVA and (P>0.05) using Tukey [12].

RESULTS AND DISCUSSION

Phytochemical test results

Phytochemical test results from a leaf of fresh *M. alba* and hexane extract of *M. alba* indicate a class of secondary metabolites respectively, steroids and terpenoids. Test results phytochemical from group fractions of A and B are triterpenoids, with red color formation after administration with Libermann-Burchard reagent. Group fractions of C and D show steroid, with green color formation after administration with Liebermann-Burchard reagent.

Characterization of isolates A1 using ^{1}H-NMR, ^{13}C-NMR, GC/MS and FTIR

The ^{1}H-NMR spectrum of A1 isolates showed chemical shifts of protons in the atom C 4-methyl chemical shift of 2.10 (3H, d, C-1); 1.28 (3H, d, C-7); 1.97 (3H, d, C-8); 2.29 (3H, s, C-15). Then the H atoms on C-metin shows chemical shift of 5.38 (1H, sept, C-3); 7.08 (1H, m, C-10); 7.08 (1H, m, C-11); 7.08 (1H, m, C-13); 7.08 (1H, m, C-14); 2.81 (1H, dd, C-6) and H atoms on C-methylene shows chemical shift of 2.77 (2H, dd, C-5).

The results of the ^{13}C-NMR spectrum of A1 isolates showed chemical shifts of 4 C atoms that bind a methyl group, 20.5 (C-1), 21.03 (C-7), 27.4 (C-8) and 20.9 (C-15). And atoms in the C-2, C-3, C-9, C-10, C-11, C-12, C-13 and C-14 indicates a shift in height is, respectively: 150.8, 124.1, 173.9, 142.5, 126.8, 129.7, 135.2, 129.7 and 126.8, this is due to the double bond that affects chemical shift higher. C atom at C-4 has a high chemical shift, due to the influence of electronegativity O atom. Chemical shifts ^{1}H-NMR and the ^{13}C-NMR isolates A1 is very similar to compounds Ar-turmerone, so a comparison between the chemical shifts of the A1 isolates with Ar-turmerone compound was done. Data comparison the chemical shift of A1 isolates with a compound Ar-turmerone can be seen in Table 1.

Table 1: Comparison of chemical shifts ^{1}H-NMR and ^{13}C-NMR, isolates from A1 ($CDCl_3$) with a compound Ar-turmerone ($CDCl_3$)

Position	^{1}H-NMR		^{13}C-NMR	
	Isolates A1	Ar-turmerone standard *	A1 isolates	Ar-turmerone standard *
1	2.10 (d)	2.10 (d)	20.5	20.7
2	-	-	150.8	155.1
3	5.38 (sept)	6.02 (sept)	124.1	124.1
4	-	-	173.9	199.5
5	2.77 (dd)	2.70 (dd)	55.3	52.7
6	2.81 (ddq)	3.28 (ddq)	35.5	35.4
7	1.28 (d)	1.23 (d)	21.03	21.9

8	1.97 (d)	1.85 (d)	27.4	27.4
9	-	-	142.5	143.7
10	7.08 (m)	7.09 (m)	126.8	126.7
11	7.08 (m)	7.09 (m)	129.7	129.1
12	-	-	135.2	135.2
13	7.08 (m)	7.09 (m)	129.7	129.1
14	7.08 (m)	7.09 (m)	126.8	126.7
15	2.29 (s)	2.30 (s)	20.9	20.9

Isolates A1 as Ar-turmerone strengthened by its MS data. MS spectrum of compound A1 in Figure 1.

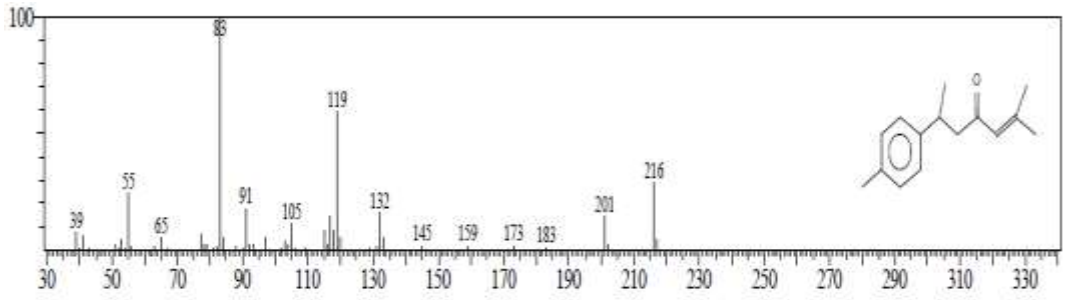

Figure 1: Patterns of fragmentation MS isolates A1

Based on Figure 1 above can be seen that the breakdown of molecular ions Ar-turmerone occur at, m/z 55, 83 and 119. The hallmarks of the compound Ar-turmerone in patterns of fragmentation are m/z 83 as the base peak [13]. A1 fragmentation pattern isolates have relatively similar fragmentation patterns with a compound Ar-turmerone. Data from FTIR, Carbonyl groups in isolates A1 shown by the peak transmittance of C=O at wave number 1735 cm^{-1}. Based on the results of characterization, with MS, ^{1}H-NMR, ^{13}C-NMR and FTIR suspected compound of isolates A1 is Ar-turmerone. The structure of the compound Ar turmerone can be seen in Figure 2.

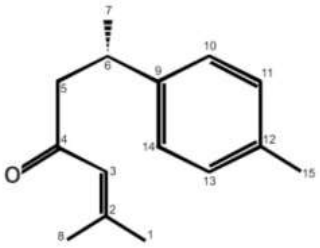

Figure 2: The structure of the compound Ar-turmerone

Hypoglycaemic activity (antidiabetic activity)

Results antidiabetic activity test in lowering blood glucose levels in mice can be seen in Figure 3 below.

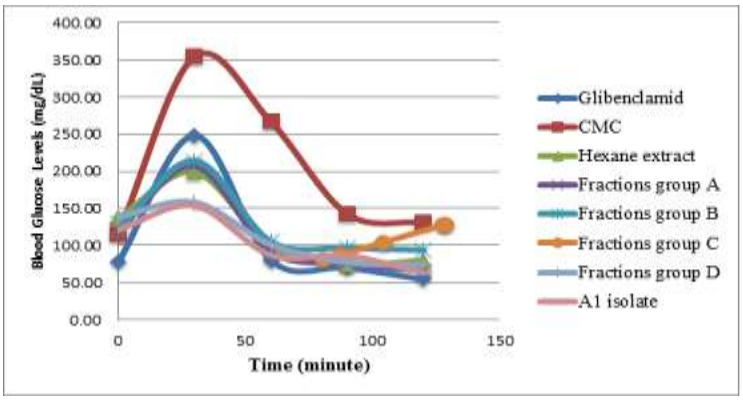

Figure 3: Graph of increase and decrease in blood glucose levels in mice against glibenclamide and CMC 1%

Based on Figure 3 shows those mice given the crude extract in 30 min to an increase in blood glucose levels into 198.67 mg/dl from 137.33 mg/dl. At min 60, 90 and 120, decreased blood glucose levels each to 100, 74.67 and 80 mg/dl. Mice were given a fraction group A at 30 min to increase blood glucose to 207.33 mg/dl of blood glucose beginning of 120.33 mg/dl, at min 60, 90 and 120 decreased blood glucose to 91.67 mg/dl, 76.33 mg/dl and 65.67 mg/dl. Mice were given a fraction B group at 30 min to increase blood glucose to 214.33 mg/dl, of initial blood glucose 122 mg/dl, at min 60, 90 and 120 decreased blood glucose to 105.33, 97 and 94 mg/dl. Mice were given a fraction group C at min 30, 60, 90 and 120 decreased blood glucose respectively are: 128, 104.33, 80.67 and 87.33 mg/dl, from the initial glucose 128 mg/dl. Mice were given a fraction group D on 30 min to increase blood glucose to 158.33 mg/dl, of blood glucose beginning of 138.67 mg/dl. At min 60, 90 and

120 decreased blood glucose respectively are to 101, 77.33 and 73.33 mg/dl. Mice were given A1 isolates at min 30, increased blood glucose to 153.67 mg/dl, of blood glucose levels early 121.33 mg/dl. At min 60, 90 and 120 blood glucose decreased respectively to 87, 86.33 and 63.33 mg/dl. Mice were given a positive control on 30 min to increase blood glucose to 248 mg/dl, of blood glucose levels early 77.33 mg/dl, at min 60, 90 and 120 blood glucose decreased respectively to 80, 70 and 54.33 mg/dl. Mice were given a negative control in 30 min to an increase in blood glucose levels into 354.33 mg/dl, of blood glucose levels early 115.67 mg/dl. Furthermore, at min 60, 90 and 120 decreased blood glucose respectively are 267, 142.67 and 130.67 mg/dl. To see the liveliness of the crude extract, group fraction A, fraction group B, group fraction C, fraction group D and isolates A1 in lowering blood glucose, to do subtraction between blood glucose of mice in negative control that with the samples at the same time. Graph result of a reduction in blood glucose levels can be seen in Figure 4.

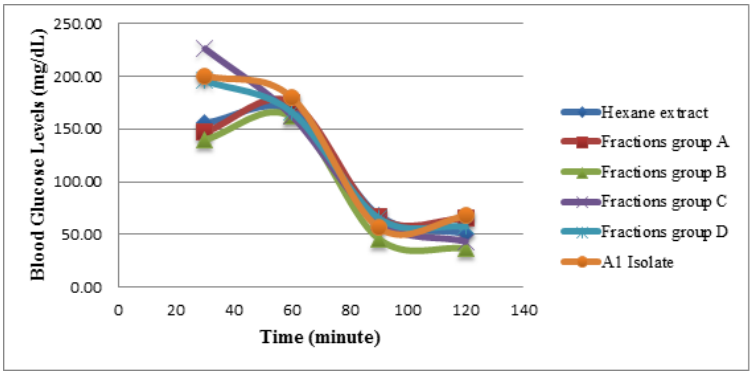

Figure 4: Results of blood glucose reduction of negative control mice with, blood glucose, mice given a crude extract, fraction group A, fraction B group, faction group C, group fraction D and isolates A1

Based on Figure 4, can be seen the activity of the samples in lowering blood glucose. The extract n-hexane, fraction group A, fraction B group, faction group C, fractions group D and compound A1 respectively showed a decrease in blood glucose levels of mice on the 30th min as many as 155.67, 147.00, 140, 226.33, 196 and 200.67 mg/dl. At min 60, the consecutive samples lowering blood glucose levels in mice as much as 167, 175.33, 161.67, 162.67, 166 and 180 mg/dl. In the 90th min, the consecutive samples capable of lowering blood glucose levels in mice by 68, 66.33, 45.67, 62, 65.33 and 56.33 mg/dl. At min 120, the consecutive samples capable of lowering blood glucose levels in mice as much as 50.67, 65, 36.67, 43.33, 57.33 and 67.33 mg/dl.

To see the difference lowering in blood glucose in Swiss Webster mice with control negative and positive is to use the Program Statistical Product and Service Solution (SPSS), the one-way ANOVA analysis wears Post hoc analysis using Tukey, in order to obtain Table 2 below.

Table 2: Differences between the blood sugar decrease, in mice, were given of crude extract, fraction group A, group of fraction B, group C fraction, fraction group D, and isolates A1, with concentration of 50 mg/kg BW, with the blood sugar decrease, in mice, were given of glibenclamide to the positive control and negative control CMC 1%, with confidence level 95% (P<0.05)

Group	Blood glucose levels (mg/dl)							
	30 min	p	60 min	p	90 min	p	120 min	p
Glibenclamide	248.00	- -	80.00	- -	70.00	- -	54.33	- -
CMC 1%	354.33	-106.333, -	267.00	-187,000* -	142.67	-72.667 -	130.67	-76,333* -
Hexane extract	198.67	49.333 (155,667*)	100.00	-20.000 (167,000*)	74.67	-4.667 (68.000)	80.00	-25.667 (50.667)
Fractions group A	207.33	40.667 (147.000)	91.67	-11.667 (175,333*)	76.33	-6.333 (66.333)	65.67	-11.333 (65,000*)
Fractions group B	214.33	33.667 (140.000)	105.33	-25.333 (161,667*)	97.00	-27.000 (45.667)	94.00	-39.667 (36.667)
Fractions group C	128.00	120.000 (226,333*)	104.33	-24.333 (162,667*)	80.67	-10.667 (62.000)	87.33	-33.000 (43.333
Fractions group D	158.33	89.667 (196,000*)	101.00	-21.000 (166,000*)	77.33	-7.333 (65.333)	73.33	-19.000 (57,333*)
A1 isolates	153.67	94.333 (200,667*)	87.00	-7.000 (180,000*)	86.33	-16.333 (56.333)	63.33	-9.000 (67,333*)

Information: *=Significantly difference from the control (P<0.05), the first line of the positive control, line 2 to the negative control

Based on Table 2 above, lowering of blood glucose by the crude extract in mice significantly different from the negative control at 30 and 60 min. Decreasing blood sugar fraction Group A in mice is significantly different from the negative control at min 60 and 120. The decline in sugar by fractions group B blood, in mice, significantly different from the negative control at 60 min. The decline in blood sugar of fraction group C in mice is significantly different from the negative control at 30 and 60 min. The decline in blood sugar by each fraction Group D and A1 isolates in mice is significantly different from the negative control at min 30, 60, and 120. Based on these differences are most active isolates A1 and group fractions of D. All samples are not significantly different from the positive control, glibenclamide, (P<5%), which states the compound is active in lowering blood sugar.

CONCLUSION

Based on the results of this study concluded that: n-hexane extract of leaves of *M. alba* positive contain secondary metabolites of steroids and terpenoids. The results of the characterization of compounds A1 has similarities with the compound of Ar-turmeron. Statistically, isolates A1 and group fraction D is the most active lowers blood glucose in mice Swiss Webster, compared with blood glucose, of mice control negative,

and all samples are not significantly different from the positive control, glibenclamide, with a level of 95% ($P<0.05$), who stated these compounds are active in lowering blood sugar.

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