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Antidiabetic Activity of ARA (*Ficus racemosa*) from Aceh

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ABSTRACT

Isolation of chemical compounds from hexane extract of the stem bark of *Ficus racemosa*, starting by maceration of 3 kg of bark *F. racemosa*, and obtained 68 g of extract concentrated, then separated by gravity column to obtained 4 groups of fraction (A, B, C and D). Separation of the group fraction A was obtained isolates A4, allegedly α -amyirin dodecanoic, which is based on spectroscopy by, Fourier Transform Infrared Spectroscopy (FTIR), Proton Nuclear Magnetic Resonance (¹H-NMR) and Carbon-13 Nuclear Magnetic Resonance (¹³C-NMR) and also, Mass Spectrometry (MS). Antidiabetic activity by glucose tolerance in mice. Activity lowers blood sugar in mice at 30th min, sorted greatest to the smallest is fraction group D (200.67 mg/dl), the crude extract (99.00 mg/dl), fraction group of B (77.67 mg/dl), fraction group C (71.33 mg/dl), fraction group A (28.67 mg/dl) and isolates A4 (4.67 mg/dl). Activity lowers blood sugar in mice at 60th min, sorted greatest to the smallest is fraction group D (136.33 mg/dl), the crude extract (51.33 mg/dl), fraction group of B (12.00 mg/dl), while isolates A4 to raise blood glucose levels of 8.67 mg/dl, as well as fraction group of B and group fraction C, to raise the blood glucose respectively 39.00 mg/dl and 17.67 mg/dl. Activity lowers blood sugar in mice at 90th min, sorted greatest to the smallest, is the fraction group of D (46.33 mg/dl), isolates A4 (43.00 mg/dl), the group of fraction A (19.67 mg/dl), the fraction C group (6.67 mg/dl) and crude extract (4.33 mg/dl). Group fraction B, at the 90th min, to raise the blood glucose levels of mice of 36.33 mg/dl. Activity lowers blood sugar in mice at 120th min, sorted greatest to the smallest, respectively from fraction group of the D (44.33 mg/dl), group C fraction (29.33 mg/dl), group A fraction (25.67 mg/dl), fraction group B (12.67 mg/dl) and crude extract (3.00 mg/dl). Group D bark fraction *F. racemosa* more active than others at 30 min of treatment, the level of 95%, ($P < 0.05$).

Keywords: *Ficus racemosa*, Antidiabetic, α -Amyrin dodecanoic, Glucose tolerance

INTRODUCTION

Diabetes is a global health problem; at least 171 million people worldwide suffer from diabetes, which is characterized by a chronic metabolic disorder with a high concentration of glucose in the blood [1]. This disease affects the nervous system of the eye, narrowing of blood vessels [2], insulin resistance, obesity, hypertension, and dyslipidemia [3].

Drug needs are great, and sometimes drugs individualistic, so searching for drugs continues to be done, and one of the plants is *Ficus racemosa*. This plant is widely spread widely throughout northern India and Australia [4]. In India, this plant has long been used for the prevention, treatment, and cure of various diseases such as obesity and diabetes, jaundice, dysentery, diarrhea, inflammation [5], skin diseases, ulcers, asthma, gonorrhoea, menorrhagia, vaginal discharge, hemoptysis, urine disease and constipation [6].

F. racemosa plant contains many alkaloids, triterpenoids, steroids and flavonoids [7]. The roots of this plant contain long-chain hydrocarbon compounds, compounds triterpene lanostane, isocoumarin and compounds phytosteroid. Fruit, sap, leaves, wood and bark of *F. racemosa* the plant contains compounds triterpenoids, steroids, coumarin and phenolic esters [6]. The results showed that the fraction and β -amyirin acetate from ethyl acetate extract at a dose of 1.5 g/kg can decrease by 2.89% and 2.31% weight of mice, respectively [8].

MATERIALS AND METHODS

Plant material

The bark of *Ficus racemosa* plants (ara) was taken in the Sarah, Aceh Besar, Banda Aceh in 2015. The plant was identified at Departement of Biology, University of Sumatera Utara, Medanense, and Medan.

Spectroscopic investigation

Mass spectra were measured using a Shimadzu GC-MS QP 2010 Ultra. ^1D spectrum, $^1\text{H-NMR}$ measured in CDCl_3 solvents, by JEOL 400 MHz spectrophotometer and spectrum $^{13}\text{C-NMR}$ measured in CDCl_3 solvents by a JEOL 125 MHz spectrophotometer. 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 [9].

Extraction and isolation of terpenoids (α -amyrin acetate) from the bark of *Ficus racemosa*

The bark of *F. racemosa* 3 kg that has dried, were macerated with n-hexane for 3×24 h, then filtered, the filtrate obtained, then evaporated with a rotary evaporator and the hexane extract obtained as much as 68.50 g or 2.28%. N-hexane extract was tested its antidiabetic activity to male Swiss Webster mice and characterized with GC-MS.

Fractionation of extract hexane

A total of 35 g of concentrated n-hexane extract was fractionated using column chromatography gravity. Eluent system is done using a gradient elution with n-hexane: ethyl acetate at a ratio of 100:0; 95:0.5; 90:10; 80:20; 70:30 and 50:50. The result of the separation of n-hexane extract obtained by 135 fractions, which consists of four groups of fractions, namely fraction group A (44-45, 13.03 g); Fraction B group (46-48, 6.3 g); Fraction group C (49-52, 2.17 g); Fraction group D (53-135, 5.92 g). Fraction group of A, shaped yellow solid, as much as 13.03 g (most clean than other fractions), was chromatographed using column chromatography gravity and obtained isolates A4. Furthermore, A4 isolates characterized using, FTIR, MS, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$.

Glucose tolerance test

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 into 8 groups, each of the groups contain three of mice. Group I: Diabetic control was given Carboxymethyl Cellulose (CMC-Na) (1%), Group II: The standard drug glibenclamide was given orally at dose of 0.45 mg/kg, body weight (BW), Group III: Treated with 50 mg/kg BW (effective dose) of fraction group of A (contain A4 isolates), Group IV: Treated with 50 mg/kg BW, hexane extract of *F. racemosa*. group V: Treated with 50 mg/kg BW, of fraction group of B. Group VI: Treated with 50 mg/kg BW, of fraction group of C, Group VII: Treated with 50 mg/kg of fraction group of D, Group VIII: Treated with 50 mg/kg BW, isolate A4 [10,11]. The extract and the fraction group A, B, C and D, isolates were suspended with CMC-Na 1%.

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 30th, 60th, 90th, and 120th min after glucose loading.

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 Post hoc analysis procedures, significant differences ($P < 0.05$) using Tukey [12].

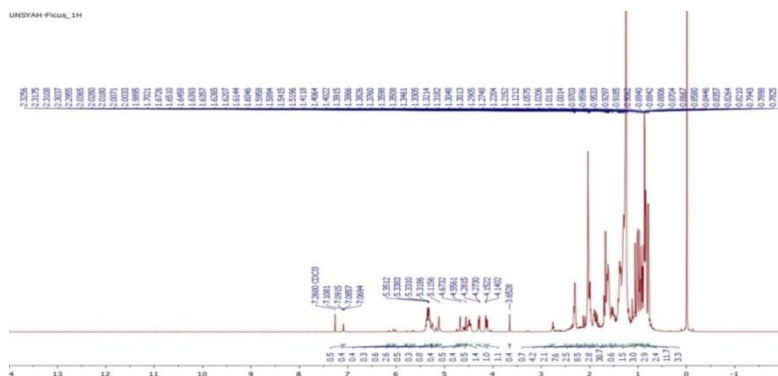
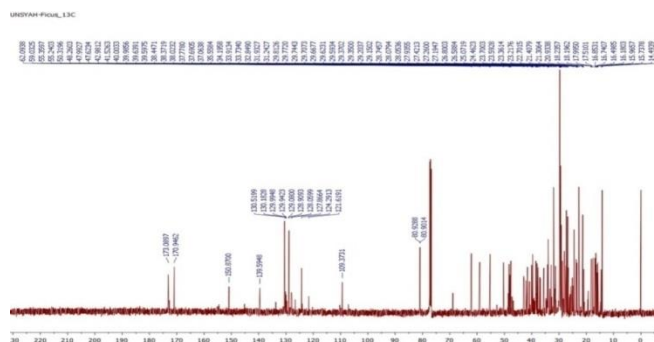
RESULTS AND DISCUSSION

Phytochemical test results from the bark of fresh *Ficus racemosa* and hexane extract of *F. racemosa* indicate a class of secondary metabolites each, steroids and terpenoids. Test results phytochemical from fractions group A, B, C and D showed that all of these fractions group contain secondary metabolites of terpenoids, which are marked with red color formation after administration with reagent Liebermann-Burchard. The isolates of A4 are also terpenoids.

Isolates A4 characterization

Isolates A4, in the form of a white solid, from hexane extract. $^1\text{H-NMR}$ spectrum for proton aliphatic (CH_3 , CH_2 , and CH) on triterpenoid compounds shown in chemical shifts (δH) 2 ppm, which is characteristic of proton cyclic of the basic framework of triterpenoids that are not separated properly [13]. Proton chemical shifts of $^8\text{C-methyl}$ at the chemical shift 0.92 ppm (s, 3H, C-23); 0.73 ppm (s, 3H, C-24); 0.79 ppm (s, 3H, C-25); 0.78 ppm (s, 3H, C-26); 1.12 ppm (s, 3H, C-27); 1.05 ppm (s, 3H, C-28); 0.87 ppm (s, 3H, C-29) and 0.84 ppm (s, 3H, C-30). One proton broad multiplet at 4.28 ppm (m, 1H) was ascribed to the protons of C-3, due to the influence of the electronegativity of O atoms and COR and presence of the olefin proton at δH at 5.11 ppm (t, 1H), characteristics of the Δ^{12} (isolated double bonds, oleanane order) [14], slider at 2.15 ppm showed ester groups. Chemical shift at 1.19 (m, 1H, C-20), shows the specific chemical shift to α -amyrin. Results $^1\text{H-NMR}$ spectrum measured in CDCl_3 contained in Figure 1.

Based on the spectrum of Figure 2, shows 44 carbon atoms that have a cluster $\text{C}=\text{O}$ with chemical shift δ is (173 ppm), ena ($\delta=139.59$ and δ 124.29 ppm). The presence of carbon shift at 124.29 ppm (C-12) and 139.59 ppm (C-13) shows very similar characteristics to α -amyrin, while the chemical shift of (C-12), 121.7-121.8 ppm and 145.2 ppm (C-13) on the β -Amyrin [15,16]. Eight C atom that binds a methyl group (C-methyl) of the compound α -amyrin that the chemical shift 27.93 ppm (C-23); 16.74 ppm (C-24); 14.49 ppm (C-25); 16.86 ppm (C-26); 23.70 ppm (C-27); 28.74 ppm (C-28); 23.36 ppm (C-29) and 21.40 ppm (C-30). Atom at C-12 and C-13 show chemical shifts are as high as 124.29 ppm and 139.59 ppm. The hallmark of the $^{13}\text{C-NMR}$ spectrum of isolates A4 shows the order triterpenoids α -amyrin [17]. The results of the characterization of isolates A4 with a $^{13}\text{C-NMR}$ instrument in Figure 2.

Figure 1: ¹H-NMR spectrum of isolates A4Figure 2: ¹³C-NMR spectrum of isolates A4Table 1: Comparison of ¹H-NMR spectral data and ¹³C-NMR isolates A4 with the compound α -standard amyirin acetate

Position H	Chemical shift (δ H) ppm (JHz)		Position C	Chemical shift (δ C) ppm (JHz)	
	A4	α - amyirin acetate*		A4	α - amyirin acetate*
1	1.27-1.35 (m)	1.28-1.35 (m)	1	38.44	38.61
2	1.67 (m)	1.67 (m)	2	24.46	24.10
3	4.28 (t)	4.43 (t)	3	80.90	79.41
4	-	-	4	38.02	38.01
5	0.84 (m)	0.84 (m)	5	55.35	55.72
6	1.54 (m)	1.54 (m)	6	18.23	19.03
7	1.38 (m)	1.38 (m)	7	23.70	23.86
8	-	-	8	41.52	41.10
9	1.54 (m)	1.54 (m)	9	48.26	48.15
10	-	-	10	37.69	37.43
11	1.62 (m)	1.62 (m)	11	18.23	18.20
12	5.11 (m)	5.12 (d)	12	124.29	125.04
13	-	-	13	139.59	140.10
14	-	-	14	42.98	43.40
15	0.86 (m)	0.86 (m)	15	29.15	29.12
16	0.79 (m)	0.79 (m)	16	37.69	37.43
17	-	-	17	33.73	33.21
18	1.98 (m)	1.91 (d)	18	59.03	59.53
19	1.00 (m)	1.00 (m)	19	40.00	40.25
20	1.98	1.98	20	38.44	38.97
21	1.30 (m)	1.30 (m)	21	31.24	31.68
22	-	-	22	41.52	40.97
23	0.92 (s)	0.93 (s)	23	27.93	27.91
24	0.77 (s)	0.73 (s)	24	16.74	16.78
25	0.79 (s)	0.80 (s)	25	14.49	14.89
26	0.78 (s)	0.77 (s)	26	16.85	16.90
27	1.12 (s)	1.10 (s)	27	23.70	23.81
28	1.05 (s)	1.05 (s)	28	28.74	28.53
29	0.87 (s)	0.87 (d)	29	23.36	23.46
30	0.84 (s)	0.84 (d)	30	21.40	21.52

*16

Based on similarity chemical shift of carbon compounds A4 with compounds α -amyrin, so isolates A4 compared with the standard α -amyrin [17]. Data comparison chemical shifts of ^{13}C -NMR isolates A4 with α -amyrin standard can be seen in Table 1.

Isolates A4 as α -amyrin, reinforced by its data of MS, MS spectrum of isolates A4 in Figure 3.

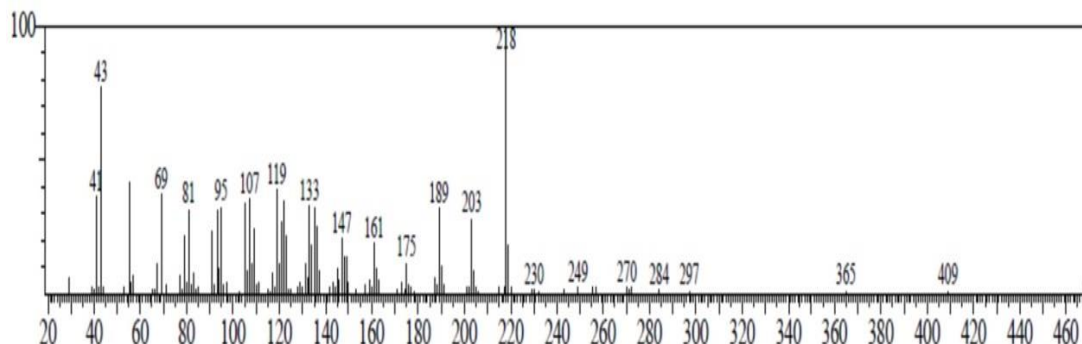


Figure 3: Mass spectrometry of the isolates A4

In mass spectrometry, fragmentation patterns isolates A4 shows the molecular ion breakdown occurs at 409, 218 (base peak), 203 and 189 m/z. Most of the triterpene compounds were found to have structural similarities with oleanane or Ursan compounds characterized by the base peak at 218 m/z, the pattern of fragmentation the molecular ions are characteristic of the α -amyrin compound, which has the same relative intensity at the peak of 203 and 189 m/z [16]. The fragmentation of 409 m/z is characteristic of α -amyrin dodecanoic [16]. So, that the isolates of A4 is a triterpene that binds ester groups. This is reinforced by spectrum Fourier Transform Infrared Spectroscopy (FT-IR). Spectrum FT-IR of A4 can be seen in Figure 4, absorbent carbonyl (C=O) of the ester at wave number 1735 cm^{-1} .

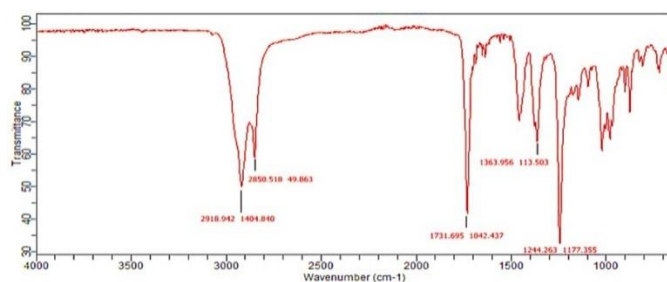


Figure 4: FT-IR spectra of isolates A4

Based on the results of the characterization of ^1H -NMR and ^{13}C -NMR, MS and FT-IR were allegedly A4 isolates is α -amyrin dodecanoic. The structure of the isolates α -amyrin dodecanoic can be seen in Figure 5.

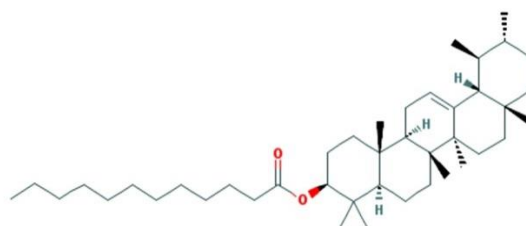


Figure 5: Structure of α -amyrin dodecanoic

Antidiabetic (Hypoglycemia) activity

Hypoglycemic activity of the extract, fractions, isolates A4, positive control and negative control, in lowering blood glucose levels in mice can be seen in Figure 6.

Based on Figure 6, above blood glucose at 30 min after induction of glucose, all groups of mice experienced an Increase in blood glucose levels. This increase is due to crude extract, fractions, A4, positive and negative controls is not yet control the sugar in the blood, so the blood sugar to rise. Blood glucose in mice given the extract crude in the 30th min increased levels of blood glucose to 255.33 mg/dl. At 60th, 90th and 120th min experienced a decrease in blood glucose levels respectively to 215.67, 138.33 and 127.67 mg/dl.

Mice were given of fraction A, at 30th min experienced an increase in blood glucose levels into 325.67 mg/dl. At 60th, 90th and 120th min experienced a decrease in blood glucose levels respectively to 255.00, 123.00 and 105.00 mg/dl. Mice were given a fraction B at 30th and 60th min experienced a rise in blood glucose levels respectively to 276.67 mg/dl and 306.00 mg/dl. In the 90th and 120th min experienced a decrease in blood glucose levels respectively to 179.00 and 118.00 mg/dl.

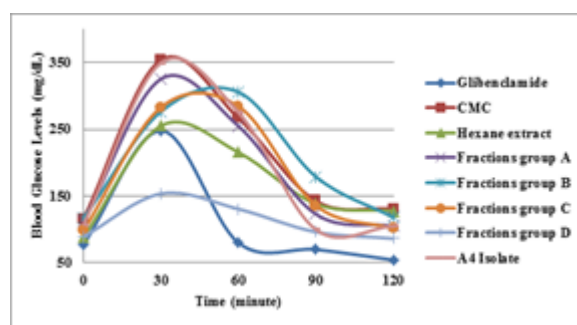


Figure 6: Graph decrease in blood glucose levels in mice against comparative glibenclamide and CMC (1%)

Groups of mice were given of fraction C at the 30th and 60th min experienced an increase in blood glucose levels respectively to 283.00 mg/dl and 284.67 mg/dl. In the 90th min and 120th experienced a decrease in blood glucose levels respectively to 136.00 mg/dl and 101.33 mg/dl. Groups of mice were given a fraction of D in the 30th min, there is increased levels of blood glucose to 153.67 mg/dl. At 60th, 90th and 120th min experienced a decrease in blood glucose levels respectively to 130.67, 96.33 and 86.33 mg/dl.

Groups of mice were given isolates A4 in the 30th min, there is an increased level of blood glucose to 349.67 mg/dl. In the 60th and 90th min experienced a decrease in blood glucose levels respectively to 275.67 mg/dl, 99.67 mg/dl but in the 120th min, there is an increased glucose level to 105.00 mg/dl.

Positive and negative control group at 30th min after treatment also showed a rise in blood glucose levels respectively to 248.00 mg/dl and 354.33 mg/dl. At 60th min after treatment showed a decrease in blood glucose levels respectively to 80.00 mg/dl and 267.00 mg/dl. Positive and negative control group at 90th min after treatment showed a decrease in blood glucose levels respectively to 70.00 mg/dl and 142.67 mg/dl and at 120th min after treatment showed a decrease in blood glucose levels respectively to 54.33 mg/dl and 130.67 mg/dl. Furthermore, the activity lowers blood sugar in mice is calculated by subtracting the blood glucose of the negative control mice, with the blood glucose of mice that given: crude extract, fraction group of A, fraction group of B, fraction group of C, fraction group of D and A4 isolates. Results of blood glucose reduction of the negative control group of rats with six kinds of the sample in Figure 7.

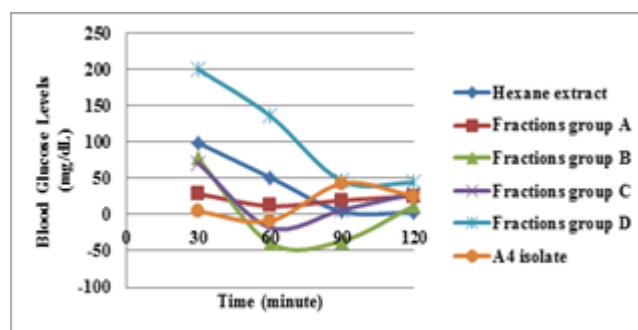


Figure 7: The result of a reduction in blood glucose in mice negative control group with blood glucose in rats that were given: crude extract, fraction group of A, fraction B group, fraction group C, group fraction D and isolates of the A4

Based on Figure 7, it can be seen that the result of reduction in blood glucose levels of negative control with group given the sample at 30th min, sorted from the greatest to the smallest is fraction group D (200.67 mg/dl), hexane extract (99.00 mg/dl), fraction group of B (77.67 mg/dl), group fraction C (71.33 mg/dl), the fraction group A (28.67 mg/dl) and isolates A4 (4.67 mg/dl).

At 60th min, the result of a reduction in blood glucose negative control mice with mice that were given samples, sorted from the greatest to the least is as follows: group fraction D (136.33 mg/dl), the crude extract (51.33 mg/dl) group fraction A (12.00 mg/dl). Isolates A4, raise blood glucose levels as much as 8.67 mg/dl and also fraction group of B and group fraction C, raise blood glucose respectively 39.00 mg/dl and 17.67 mg/dl.

At 90th min, the result of a reduction in blood glucose negative control mice with mice that were given samples, sorted from the greatest to the least is as follows, the fraction D group (46.33 mg/dl), isolates A4 (43, 00 mg/dl), fraction group A (19.67 mg/dl), the fraction C group (6.67 mg/dl) and crude extract (4.33 mg/dl). Groups of mice were given a fraction B at 90th min to raise the blood glucose levels of 36.33 mg/dl.

At 120th min, the result of a reduction in blood glucose negative control mice with mice that were given samples, sorted from the greatest to the least is as follows, the fraction D group (44.33 mg/dl), group C fraction (29.33 mg/dl), fraction group A (25.67 mg/dl), group fraction B (12.67 mg/dl) and crude extract (3.00 mg/dl).

Based on the above, generally all groups of fractions extract n-hexane and isolate A4, the bark of fig plants (*F. racemosa*), are relatively active in lowering blood glucose levels. To see the difference in the activity of lowering of blood sugar in mice Webster male among the 6 types of samples above, (crude extract, group fraction A, group fraction B, group fraction C, fraction group of D, and compound of A4), is to use the Program Statistical Product And service Solution (SPSS), the analysis with ANOVA one-ways Post hoc analysis using Tukey, in order to obtain Table 2.

Group D fraction, significantly different from the negative control, and other samples at the time of 30 min.

Table 2: Comparison of blood glucose levels decrease, crude extract, group fraction A, group fraction B, group fraction C, group fraction D and isolates A₄, with a concentration of 50 mg/kg bb of mice and the P-value (0.05) against glibenclamide (control positive) and CMC 1% (negative control)

Groups	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	255.33	-7.333	215.67	-135.667	138.33	-68.333	127.67	-73.333*
Fractions group A	325.67	-77.667	255.00	-175.000	123.00	-53.000	105.00	-50.667
Fractions group B	276.67	-28.667	306.00	-226.000	179.00	-109.000	118.00	-63.667
Fractions group C	283.00	-35.000	284.67	-204.667	136.00	-66.000	101.33	-47.000
Fractions group D	153.67	94.333	130.67	-50.667	96.33	-26.333	86.33	-32.000
A ₄ isolate	349.67	-101.667	275.67	-195.667	99.67	-29.667	105.00	-50.667
		4.667		-8.667		43.000		25.667

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

CONCLUSION

The test results antidiabetic n-hexane extract from the bark of fig plants (*F. racemosa*) have an activity to lower blood glucose levels in mice. Group D bark fraction *F. racemosa* more active than others at 30 min of treatment, the level of 95%, (P<0.05). The results of the characterization of compounds A₄ have similarities with the compound of α -amyrin dodecanoic.

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