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Isolation Compound Anti-obesity from the Bark Ara (*Ficus Racemosa*) of Aceh

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ABSTRACT

The extract n-hexane and ethyl acetate obtained from plants *Ficus racemosa*, respectively of 68.50 g (2.28%) and 50.52 g (1.68%), were tested their antiobesity, with dose of 100, 500, 1000 and 1500 mg / kg bw of mice. The results showed that the extract n-hexane and ethyl acetate can weight loss in mice, obtained optimal dose is 1500 mg / kg bwof mice and used as dosis of next work. Furthermore, 30 g of ethyl acetate extract were fractionated, and yielding three groups of fractions, namely fraction groups A, B and C.Test antiobesity against the fractions A, B, and C were carried out for 5 days each using three mice, can decrease mice weight of average : 2.89%; 2.57%; 0.56% while 4.14% for positive control, whereas the negative control mice increase weight of mice as much as 4.82%. Fraction A, after separated with chromatography produce a compound suspected of β-amyrin acetate. Compounds β-amyrin acetate at a dose of 1500 mg / kg bw of mice, showed a weight loss of 2.31%. β-amyrin acetate compound was characterized by Mass Spectrometry (MS), Proton Nuclear Magnetic Resonance (^{1}H-NMR) and Fourier Transform Infrared Spectroscopy (FT-IR).

Keywords: anti-obesity, *Ficus racemosa,* isolation, β-amyrin acetate.

INTRODUCTION

Obesity (overweight) is one disease that often occurs among children, adolescents and adults. According to the International Obesity Task Force (IOTF) that more than 1.1 billion adults worldwide are overweight and 312 million are obese. Obesity is very dangerous because it can cause a wide range of other diseases such as diabetes, heart disease, stroke and certain types of cancer[1].

One of the plants that have the potential in the medical field is *F. racemosa* of the family Moraceae, which is used in the system of traditional medicine for diarrhea, dysentery, hemorrhoids, wound healing, skin disorders, diabetes, ulcers,

impaired bile, anti bleeding, colds, antioxidants and as well as agents anticancer[2]. The results reported, suggested that *F.racemosa* compounds containing tannins, saponins gluanol acetate, betasitosterol, lupeol, lupeol acetate and β-amyrin acetate[3]. Leaf extract of Ficus racemosa Linn. active as an anti-bacterial of *Escherichia coli*, *Basillus pumilis*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*[4].

MATERIAL AND METHODS

Plant Material

The bark of *F. racemosa* was collected in February 2015 in Aceh, Indonesia. The plant was identified at Department of Biology, University of North Sumatera, Medanense, Medan.

Animal

All experiment were carried out using breeding 4-6 week old male Swiss Webster mice chosen from animal colony of central animal research facility, University of North Sumatera, Medan. The colony was maintained under controlled condition soft temperature.

Spectroscopic Investigation

IR spectrum was found using a Perkin Elmer Spectrum One FT-IR spectrophotometers. Mass spectra were measured using a Shimadzu GC-MS QP 2010 Ultra. The ^{1}HNMR (400 MHz), Spectrum were recordedon a JEOL in CD_3Cl.

Testing Phytochemicals

The method used for testing of phytochemicals can be found in: Phytochemical methods, Simplified Determination Method to Analyze plant[5]

Extraction And Isolation of β-Amiryn Acetate From The Bark Of *F. Racemosa*

The air dried of bark of *F. racemosa*(3 kg) was extracted with hexane for two times in percolator and filtered. The filtrate was evaporated in vacuo to give the dark brown residue, and the yield was 68.50 g. Then the sample was also extracted with ethylacetate solvent, and the yield was 50.52 g (extract of ethylacetate). All of these extract were collected separately and preserved for bioassay test and analysis. Antiobesity test results from both of the extracts, the ethyl acetate extract slightly more active from hexane extract, so the isolation and testing activities was directed at the ethyl acetate extract.

Ethylacetate Extract Fractionation

The ethyl acetate extract (30 g) was separated by column chromatography with silica gel (70-230 mesh, 150 g, Merck) as stationary phase and eluted with n-hexane gradually with ethylacetate acetate (100: 0; 98: 2; 95: 5; 90:10; 80:20), to efford 86 fractions by TLC profile.

Fractions (1-16, A) as much as 7.01g, contained terpenoid, fractions (17-34, B) as much as 9.8 g, contained steroid, and fractions (35-86, C) as much as 1.55 g, contained terpenoids. Fraction

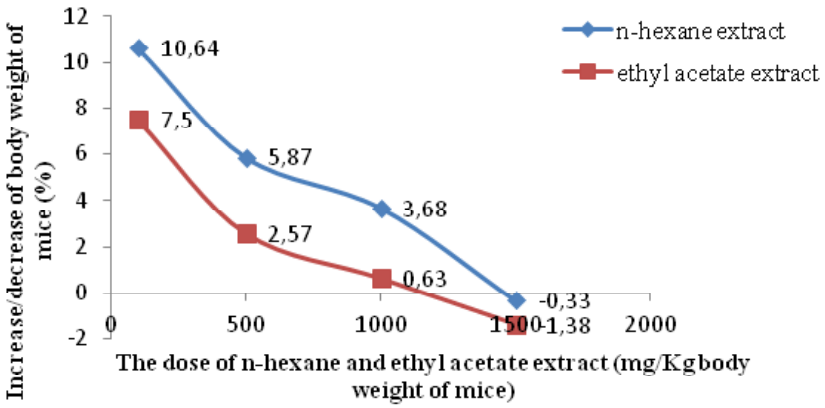

Fig. 1: Curves of reduction average weight of mice with different doses of the extract n-hexane and ethyl acetate (for 5 days)

1-16 (3 g) was further fractionated using column chromatography (70-230 mesh, 100 g, Merck) eluting with eluent system of n-hexane: ethyl acetate (98: 2; 95: 5; 90:10) to obtain a yellowish white solids were 250 mg (A4). This solid was further fractionated using column chromatography (70-230 mesh, 15 g, Merck) using a system of eluent n-hexane: ethyl acetate (98: 2; 95: 5; 90:10),to obtained a white solid ($A4_4$). Furthermore, this pure isolate was tested its antiobesity activity and characterized using ^{1}H-NMR and FT-IR.

Antiobesity activity test[6]

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. Having fasted for 20-24 hours , the weight of mice were weighed, then mice were divided into 5 groups, each of the groups contain three of mice, group I: obesity control (negative control is given food (pellets) without medication, no given extract or xenical), group II: treated with standard drug, xenical, was given orally at dose of 1500 mg/kg bw of mice), group III, IV, and V: treated with extract of F. racemosa, with dosis 1500 mg/kg bw of mice. Weight measurement is done by weighing the mice every day in five days[7].

RESULTS AND DISCUSSION

Phytochemical Test Results

Phytochemical test results of fresh bark *F. racemosa* and the ethyl acetate extract of *F. racemosa* showed a class of secondary metabolites, respectively, steroids, terpenoids and saponins.

Antiobesity activity

The test results antiobesity of extract n-hexane and ethyl acetate to mice can be seen in Figure 1.

Based on Figure 1 shows that mice fed an crude extract n-hexane and ethyl acetate of with a dose of 100 mg / kg bw of mice, the weight

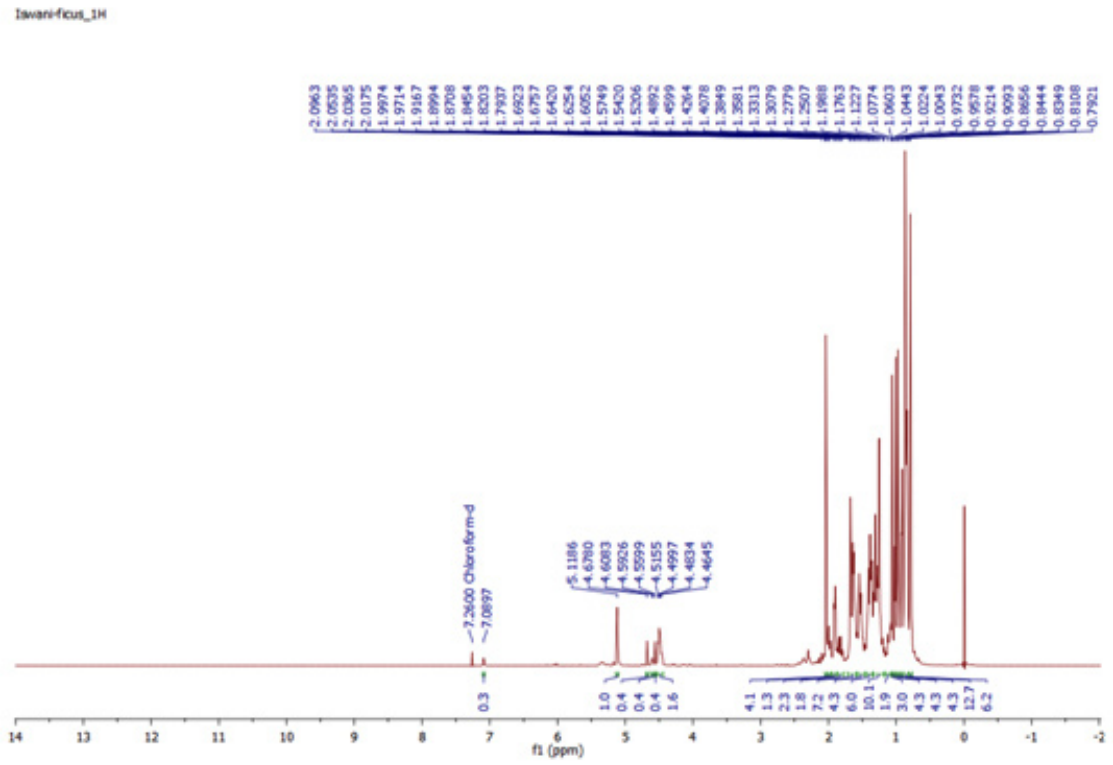

Fig. 2: ^{1}H-NMR spectrum of Compound $A4_4$

on average increased, respectively by 10.64% and 7.50% , weight is risen because a given dose of the extract is still very low. Mice were given extracts of n-hexane and ethyl acetate at a dose of 500 mg / kg bw of mice, weight of mice increased relatively minor, respectively: 5.87% and 2.57%. Mice that were fed n-hexane crude extract and ethyl acetate extract at the dose of 1000 mg / kg bw of mice, increased weight is relatively smaller, respectively 3.68% and 0.63%. Mice that were fed n-hexane crude extract and ethyl acetate extract rough at doses 1500 mg / kg bw of mice, body weight of rats was reduced by respectively 0.33% and 1.38%. So that this dosage used as a dose of work.

Further testing anti-obesity activity, carried out against a group fraction chromatography results ethyl acetate extract (fraction groups A, B, and C).

Table 1: Results of testing the activity of anti-obesity of fractions A, B, and C (%)

Fraction	The decrease /increase of weight of mice with given fraction A, B, C for 5 days (each 3 mice) (%) Mice (I)	Mice (II)	Mice (III)	Average total (%)
fraction A	3.54	-3.03	-9.19	-2.89
Fraction B	0.16	-4.78	-3.10	-2.57
Fraction C	-1.61	-0.49	0.40	-0.56
Control (+)	-0.81	-5.86	-5.76	-4.14
Control (-)	4.48	4.84	5.16	4.82

Note: (+) weight gain in mice
(-) Weight loss in mice

Table 2: Comparison of the ^{1}H-NMR spectrum data of compound A4$_4$ ($CDCl_3$, 500 MHz) with standard of β- amyrin acetate compound

Position H	Chemical shift (δ_H) ppm (J Hz) A1	β- amirin asetat*	Position H	Chemical shift (δ_H) ppm (J Hz) A1	β- amirin asetat*
1	1.28-1.35 (*m*)	1.04 -1.64 *(m)*	16	0.79-1.91 (*m*)	0.79 – 1.98 *(m)*
2	1.67 (*m*)	1.60 – 1.88 (*m*)	17	-	-
3	4.49 (*t*)	4.50 (*t*, 8.0 Hz)	18	1.91 (*d*)	1.93 (*d*; 4.2 Hz)
4	-	-	19	1.00-1.67 (*m*)	1.00 -1.66 *(m)*
5	0.84 (*m*)	0.84 *(m)*	20	-	-
6	1.54 (*m*)	1.40 – 1.53 *(m)*	21	1.30 (*m*)	1.08 – 1.32 *(m)*
7	1.38 (*m*)	1.33 – 1.52 *(m)*	22	-	-
8	-	-	23	0.90 *(s)*	0.88 *(s)*
9	1.54 (*m*)	1.58 *(m)*	24	0.97 *(s)*	0.96 *(s)*
10	-	-	25	0.86 *(s)*	0.86 *(s)*
11	1.62 (*m*)	1.63 -1.80 *(m)*	26	1.00 *(s)*	0.97 *(s)*
12	5.11 (*t*)	5.18 (*t*; 3.5 Hz)	27	1.06 *(s)*	1.13 *(s)*
13	-	-	28	0.84 *(s)*	0.83 *(s)*
14	-	-	29	0.86 *(s)*	0.88 *(s)*
15	0.86 (*m*)	0.85 – 1.76 *(m)*	30	0.79 *(s)*	0.87 *(s)*
OCH_3	2.03 *(s)*	2.05 *(s)*			

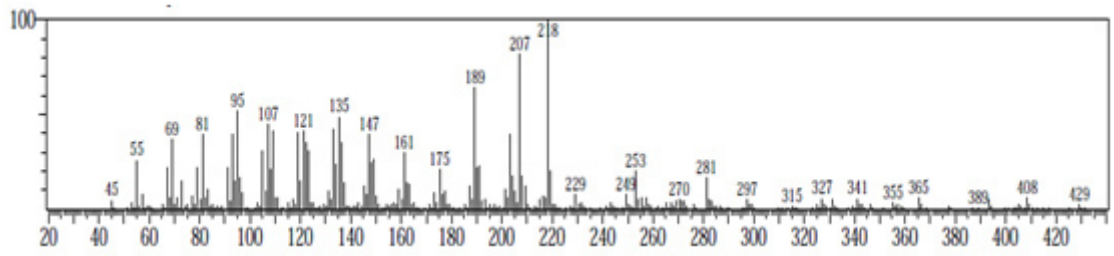

Fig. 3: Mass spectrometry compound $A4_4$

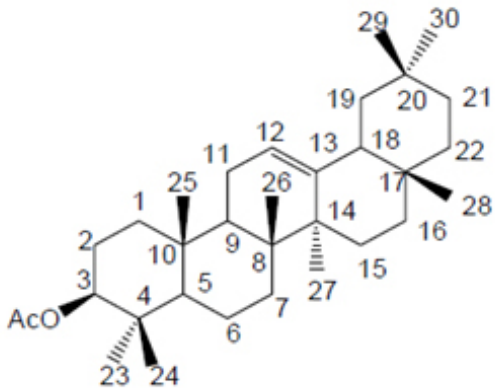

Fig. 4: β-amyrin acetate

The test results antiobesity fraction (A, B, C) of the mice are shown in Table 1.

Based on the result of a decrease / increase in the average weight of mice contained in Table 1, can be seen that all fractions A, B, and C, can reduce the body weight of mice. Fraction of A may reduce mice weight (2.89%) is greater than the fraction of B (2.57%) and the fraction C (0.56%). Positive control, xenical can lose weight of mice by an average of 4.14%, while the negative control increase the mice weight of average as much as 4.82%.

Test Antiobesity Pure Isolates ($A4_4$) On Swiss Webster Mice

$A4_4$ pure isolates can lose weight in mice as much as 2.31%, while Xenical as a positive control to lose weight as much as 4. 49%, and the weight of negative control mice rose as much as 4. 31% in the same experiment.

Determination Structure $A4_4$

$A4_4$ compound, was obtained as a colourless amorphous powder from ethilacetate extract. The ^{1}H NMR spectrum of $A4_4$ compound, measured in $CDCl_3$, Characterization of compound $A4_4$ with ^{1}H-NMR can be seen in Figure 2.

^{1}H-NMR spectrum for proton aliphatic (CH_3, CH_2 and CH) on triterpenoid compounds shown in chemical shifts (äH) 2 ppm, which is characteristic of proton cyclic of basic framework of triterpenoids that are not separated properly[8]. Compound $A4_4$ showed 8 C-methyl chemical shifts (äH ppm) 0.90 (3H, s, H-23); 0.97 (3H, s, H-24); 0.86 (3H, s, H-25); 1.00 (3H, s, H-26); 1.06 (3H, s, H-27); 0.84 (3H, s, H-28); 0.86 (3H, s, H-29); 0.79 (3H, s, H-30).

Acetyl group at chemical shifts (δH ppm) 2.03 (3H, s, H-32), one proton broad multiplet at 4.49,(m,1H) was ascribed to proton of C-3, due to the influence of the electronegativity of O atoms and $COCH_3$ and presence of the olefin proton at δH shift 5.11 ppm (t, 1H), characteristics of the Δ^{12} (isolated double bonds, order oleanan)[2].

Furthermore, determination of structure of the compound $A4_4$ done by comparing the chemical shifts (δH) between compounds $A4_4$ with compounds β-Amyrin acetate standard[9]. Data comparison of chemical shifts (δH) compound $A4_4$ with β-Amyrin asetat compounds, can be seen in Table 2.

Table 2 shows that the compound of $A4_4$ is similiar with β-amyrin acetate standard. Based on the comparison of the data of the ^{1}H-NMR, this compound is thought to contain the compounds β-amyrin acetate. Chemotaxonomic approach based

compounds β-amyrin acetate is also present in plants *A. camansi*[10], *Ficus retusa* L.variegata[11], *Dorstenia arifolia*[12], which is also a family Moraceae

The above data is reinforced with Mass Spectrometry (MS) spectrum of data, which indicate the presence of *â*-amyrin acetate compound.

From fragmentation of compound β-amyrin acetate standar, showed molecular ion [H^+] m/z:468, 218, 207, 189 and the 69. Pattern fragmentation 218 shows the breakdown $C_{16}H_{25}$ [M^+] 203 ([$CH_3M]^+$). The abundance ion at m/z 218, 203, 189, and 69 are typical for the fragmentation of β- amyrin acetate[13].

However, the MS spectrum of compound $A4_4$ looks a molecular ion at m / z 426 which constitutes the frame of β-amirin[9, 14]. This is because the group $COCH_3$ (m / z 43) was not seen in the data of MS. Characteristic of β-amyrin acetate compound contained in fragment the molecular ion m / z 218 as the base peak[13, 15]. The solving molecular ion β-amyrin compound[15]

Based on the results of data analysis and FT-IR spectrum compound of $A4_4$, shows the absorbance of carboxyl groups C-O (ester) at wave number 1241 cm^{-1}. At wave number 1366 cm^{-1} showed absorbance $C(CH_3)_2$ (gem-dimethyl), namely the existence of two methyl groups on the same carbon. At wave number 1735 cm^{-1} there is absorbance of carbonyl C = O ester ($R\text{-}CO_2\text{-}R$ ') and the wave number 2851-2919 cm^{-1} there is absorbance of C-H (alkyl). Based on comparative data ^{1}H-NMR and Mass Spectra and FT-IR of isolate $A4_4$, is suggested isolate $A4_4$ as β-amyrin acetate, which can be seen in Figure 4.

The structure of β-amyrin acetate compound can be seen in Figure 4.

CONCLUSION

Based on the results obtained can be concluded that the test results showed *F. racemosa* extract contains secondary metabolites, terpenoids and steroid group. The crude extract ethyl acetate at a dose of 1500 mg / kg bw of mice, can lose weight by 1.38%. All fractions A, B, and C, can reduce the body weight of rats. Fraction of A may reduce weight of mice as much as (2.89%) is greater than the fraction of B (2. 57%) and the fraction C (0.56%), while $A4_4$ pure isolates can lose weight in mice as much as 2.31%.

Characterization of isolates $A4_4$ with ^{1}H-NMR, IR and mass spectra, allegedly fraction of $A4_4$ is β-amyrin acetate.

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REFERENCES

1. Haslam D.W, James W.P. *Lancet*, **2005**, *366* (9492): 209-1197,
2. Rawat, P.;Rawat, P.; and Kumar, P. *Journal Of Drug Discovery And Therapeutics,* **2013***1* (1), 15-18
3. Devaraj, K.B.; Gowda, L.R. and Prakash, V. *Phytochemistry*, **2008,** *69*(3): 647-655.
4. Mandal, C.S; Saha, B.P.; Pal.M. *Phytotherapy Research* , **2000**, 14(4): 278–280
5. Harborne, J. B. Phytochemical Methods, **1987**, second edition, London EC4P4EE.
6. Triplitt, C.L., Reasner, C.A., and Isley, W.L. Diabetes Mellitus *in* editors Dipiro, J. T., Talbert, R.L., Yee, G.C., Matzke, G.R., Wells, B.G., Posey, L.M. *Pharmacoterpy A Pathophysiologic Approach***, 2005**, Sixth Edition, 1335, The McGraw-Hill Companies, New York,
7. Fajriaty, I.Tesis, **2014**,. Institut Teknologi Bandung, Bandung,
8. Deny, R., dan Puji, A. J.K.K. **2013,** *2*(1): 7-12,
9. Shan, Y..L.; Thing, C.T.; Ping, S.T.; Awang, K.; Hashim, M. N.; Nafiah, A. M. and Ahmad, K.

J. Chem. Pharm. Res., **2014**, *6*(5):815-822,
10. Nasution, R., Marianne., Nur, H. *Der Pharma Chemica*, **201**5, *7*(6):71-78.
11. Sarg, M. T.; Abbas, A.F.;Taha; El-Sayed, I.Z. and Mustafa, M.A. *Journal of Pharmacognosy* and *Phytotherapy* , **2011**,. *3*(7), 89-100.
12. Fingolo, E.C.; Santos, S.T.; Filho, V. M.D.M.and Kaplan, C. A. M. *Molecules* **2013**, *18*, 4247-4256; doi:10.3390/molecules18044247
13. Fernandes, C.P., Corrêa, A.L., Lobo, F. R., Caramel, O.P., Almeida, F.B., Castro, E.S., Souza, F.C., Burth, P. *BioMed Research International*, **2013,** 1-7.
14. Bahuguna, Y., Chakraborthy, G.S. *World Journal of Pharmacy and Pharmaceutical Sciences*, **2014**, *3*(10): 1168-1188.
15. Sigrid, K.K. Thesis, **2009,** University of Bergen, Norway.