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Anti-Obesity Compounds from the Leaves of Plants *Morus Alba* (MORACEAE)

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Abstract: Morus alba leaves as much as 2 kg, was macerated with hexane and ethyl acetate solvent, hexane extract obtained 79.33 g, and 54.31 g of ethyl acetate extract, test antiobesity against both these extracts, namely by providing hexane extract and the ethyl acetate extract to the male Swiss Webster mice at various concentrations, starting with a concentration of 100; 500; 1; 1.5; and 3 grams. Mice were weighed every day, and seen changes in weight. The results showed that both types of this extract can reduce the weight of mice, but anti-obesity activity of the ethyl acetate extract relatively greater compared to the hexane extract. The optimal dose for the ethyl acetate extract is 1.5 g/ bw mice, the mice lose weight as much (-335g).
Furthermore, this dose is used to test anti-obesity of group fraction of the fractionation of the ethyl acetate extract. The test results of group A fraction can reduce the weight of mice with an average of -1.098 g; Fraction B group can lose weight mice with an average of -1.025 g; Fraction group C can reduce the weight of mice with an average of -0.83 g;.
Fraction Group D, was raise up the weight of mice with an average of 0.92 g; Xenical compound (positive control) can lose weight of mice by an average of -1.098 g; and a negative control (untreated) can lose the weight of mice by an average of 0.85 g.
The characterization results extract of ethyl acetate by GC-MS, is known to contain: Ar-tumerone (1); Tumerone (2); Alpha-Tumerone (3); Dibutyl phthalate (4); and the compound steroid (5), and others.
Key words: *Morus alba*, anti-obesity, mice Swiss Webster, tumerone.

Introduction

Obesity is a huge problem in the world,, Ten percent, or about 155 million young people in the world are overweight (obese). Chairman of the Public Nutrition at the London School of Hygiene and Tropical Medicine, called for immediate action. Epidemics also occur in children, which used to be considered adult problems. Obesity causes the chances of various diseases, as: heart disease, type 2 diabetes, obstructive sleep apnea, certain cancers, osteoarthritis [1]

Searching in the literature it is known that plants *Morus alba* (Family Moraceae) of the genus Morus, contains many chemical compounds have properties, such as: drugs flu, malaria, hypertension, asthma, diabetes, insomnia, vertigo, anemia, hepatitis and diabetes mellitus

Morus alba plants contain compounds betasitosterol and lupeol, which can help the metabolism of fat and cholesterol, and can also prevent excessive absorption of cholesterol in the intestine to enter the bloodstream [2].

So this research aims to find a compound that works to lose weight male Swiss Webster mice, and the concentration of the compound to lose the mice weight**.**

Materials and Methods

This research was conducted at the Research Laboratory of the Department of Chemistry, UNSYIAH Banda Aceh in 2015.

Plant Material

Leaves of plants *Morus alba* (mulberry) was taken in the Village Miruk, District Krueng Barona Jaya, Aceh Besar, Banda Aceh in 2015. The plant was identified at the Department of Biology, University of North Sumatra, Medan**.**

Extraction and isolation of compound from the leaves of *Morus alba*

Isolation Extract from the leaves of *Morus alba* by maceration with hexane solvent, for 3x24 hours, then filtered, the filtrate obtained is evaporated with a rotary evaporator, and the hexane extracts obtained as much as 79.33 g. Furthermore, its pulp macerated again with the solvent ethyl acetate, and the ethyl acetate extract is obtained as much as 54.31 g.

Both types of extracts can lose weight in mice, but the extract ethyl acetate relatively more active, so testing is focused on the ethyl acetate extract.

Fractionation Ethyl acetate Extract

Ethyl acetate extract of 30 grams was separated by using column chromatography(gravity chromatography) with gradient of elution with eluent system n-hexane: ethyl acetate of 100: 0; 90:10; and 95:5. This separation yield 84 fractions, after monitored by thin-layer chromatography (TLC), the same stain pattern was grouped, and obtained 4 group of fractions (A, B, C, D). Group of fraction A (32-42) as much as 8.22 g; B (44-59) as much as 1.34 g; C (61-68) as much as 0.64 g, and D (71-84) as much as 1.26 g.

The group of fractions A, B, C, and D were tested their activity to antiobesity of male Swiss Webster mice

Characterization Of Chemical Compounds In The extract of Ethyl Acetate

Characterization of the ethyl acetate extract using gas chromatography-mass spectrometry (GC-MS), conducted in the laboratory of Instrument at the university North Sumatra, Medan. [GCMS-QP2010 Plus]

Ion Source Temp :250.00 °C
Interface Temp. :320.00 °C
Solvent Cut Time :2.00 min
Detector Gain Mode :Relative
Detector Gain :0.00 kV
Threshold :1000

Test Anti-Obesity

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.

Animals were grouped into 5 groups

I: Given the food and were not given the drug

II: III; IV: given food coupled with the ethyl acetate extract, at a dose of 1.5 g / body weight of mice (the optimal of dose extract etlasetat).
V: Given the anti-cholesterol drugs (eg Xenical)

Animal testing is a male Swiss Webster mice, the mice was given food plus extract every day for 5 days. Weight of mice were weighed every day. Weighing is also done before the observation, the amount of food is weighed every day.

Testing Phytochemicals

The method used for testing of phytochemical can be found in: Phytochemical methods, Simplified Determination Method to Analyze Plant [3]

Results and Discussion

Testing Phytochemicals

Phytochemical test *Morus alba* leaves obtained types of secondary metabolites: terpenoids and steroids.

Determination of optimum doses of extracts Ethyl Acetate For Anti-Obesity

Ethyl acetate extract obtained from plants *Morus alba*, tested its anti-obesity activity using male Swiss Webster mice were about 2 months old, and healthy. Results of testing anti-obesity activity of ethyl acetate extracts of *Morus alba* leaf contained in Figure 1 below.

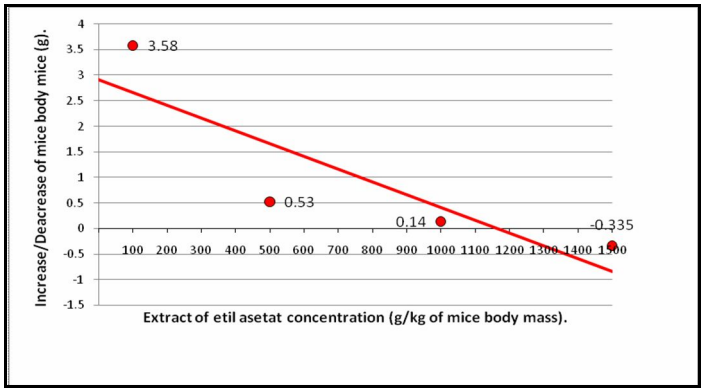

Figure 1. Graph increase / reduction in body weight of mice with varying concentrations of ethyl acetate extract (every 3 days)

Signs -: reduce weight

Based on Figure 1 it can be seen that the use of ethyl acetate extract as much as 100 mg / bw of mice led to raise up an average weight as much as 3.58 g mice, similarly with it, to a concentration of 500 mg and 1 g, the weight of mice increased respectively 0.53 g and 0.14 g. At a concentration ethyl acetate extract of 1.5 g / bw of mice , caused a decrease in the average body weight of mice as much as -335 mg, so this dose was used to test antiobesity next mice.

Anti-obesity test to group of fraction A, B, C, and D

Phytochemical test, group of fractions A, B, C, is inactive against to reagent, of Lieberman Burchard which shows this compound is not triterpene or steroid, the possibility of monoterpenes. Group of fractions D, shows the turquoise color with Lieberman Burchard reagent which indicates steroid compounds. The Four groups of fractions was tested its anti-obesity activity. The test results of the group of fractions to antiobesity of the extract of ethyl acetate in Table 1 below

Table 1. Addition / Reduction Weight average Mice fed a diet plus a group of fractions A, B, C, and D / Control Positive / Negative Controls (5 days) and body weight of mice Average Total

The group type of Fraction / positive control / negative control	Reduction / addition of Weight Average Each mice Over 5 Days			Reduction / addition of Weight Average total mice
	Mice 1	Mice 2	Mice 3	
Feeding with a group of fractions A	1,3025	-3,6325	-0,9666	-1,098
Feeding with a group of fractions B	-0,2925	-1,73	-1.0525	-1,025
Feeding with a group of fractions C	-1,205	-0,2	-1,085	-0,83
Feeding with a group of fractions D	3,175	-0,3687	-0,043	0,9209
Positive Control	-0,5425	-2,17	-0,2925	-1,3565
Negative Control	0,4875	1,505	0,5575	0,85

Based on Table 1 above it can be seen that the group of fractions A, B, C, can lose weight male Swiss Webster mice significantly, while granting group of fraction D, was increased weight of mice. A positive control is the administration of drugs that have been sold commercially, Xenical, can reduce the greatest weight (-1.3565 g with the same dose with a group of fractions), and a negative control (only given food), raise the weight of mice (0 , 85 g).

Weight loss in mice that were fed a group of fractions A, B, and C is caused by the presence of compounds: Ar-tumerone or 2-Methyl-6- (4-methylphenyl) -2-hepten-4-one (similiarity 86%) (**1**); Tumerone or 2-Methyl-6- (4-methyl-1,3-cyclohexadien-1-yl) -2-hepten-4-one, (similiarity 89%) (**2**); Alpha.-Tumerone (similiarity 90%) (**3**); Dibutyl phthalate or 1,2-Benzenedicarboxylic acid, dibutyl ester or Phthalic acid, dibutyl ester or n-butyl phthalate (similiarity 97%) (**4**); which can reduce cholesterol in the body of mice.[4] [5]

Compounds of this turmeron always associated with family Zingiberaceae plants [6] such as saffron. Turmeron in turmeric extract may affect transport of curcumin to the cells of the intestine that causes increased absorption of curcumin[7]. Prathapan (2012), states that turmeric extracted with ethanol can be used as a safe anti-obesity drugs [8]. Compounds of turmeron in the volatile oil in turmeric can inhibit the growth of fat tissue [9].

Based on the above, it can be said turmeron compounds in plants Morus alba has the same activity with tumeron the compounds in the plant of turmeric

While D, a steroid compound, proved of phytochemical test with Lieberman Burchard produce green color and spectrum MS (from GC-MS) that resembles Cholesterol 3-O - [(2-acetoxy) ethyl (similiarity only 64%), but it is not known exactly the type of steroid.

MS spectra of compounds turmeron and comparison (of GC-MS) were obtained as follows:

Compound 1.

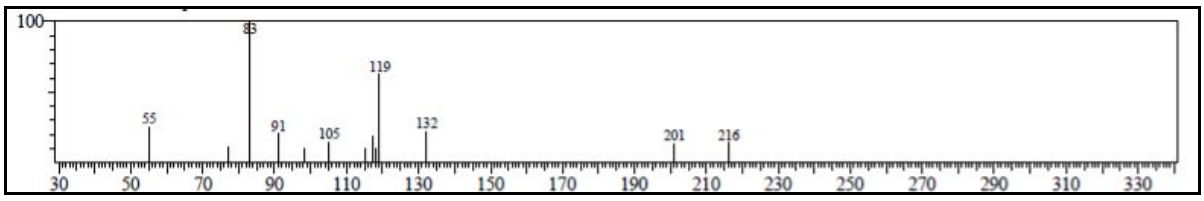

Figure 2. Spectrum of unknown compounds (of GC-MS) Comparative spectrum is as follows:

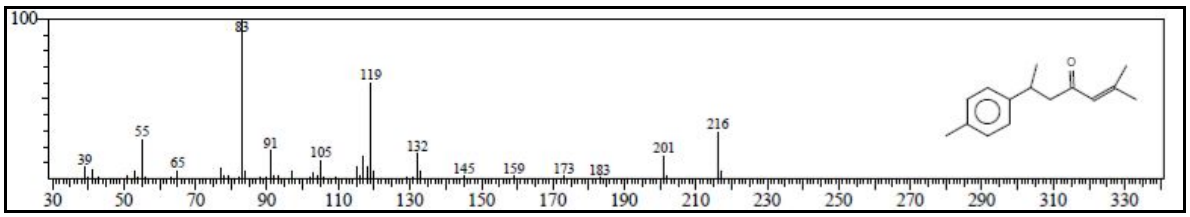

Figure 3. Spectrum of the compound Ar-turmerone

Compound 2.

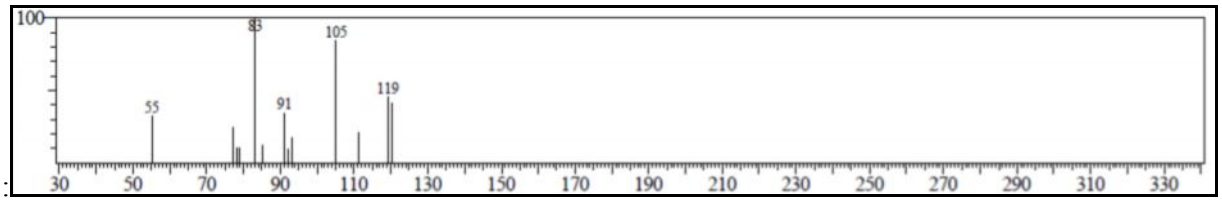

Figure 4. Spectrum of unknown compounds (of GC-MS) Comparative spectrum is as follows:

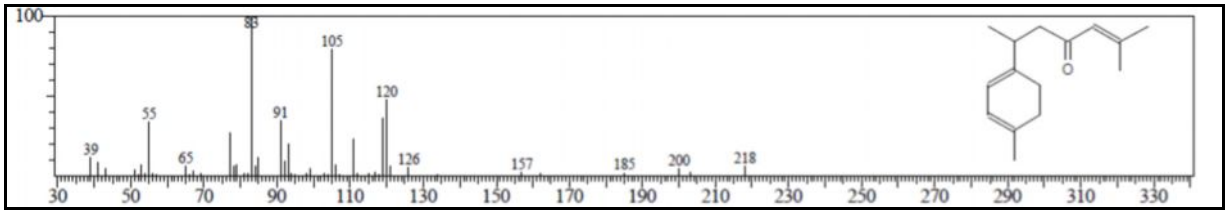

Figure 5. Tumerone

Compound 3.

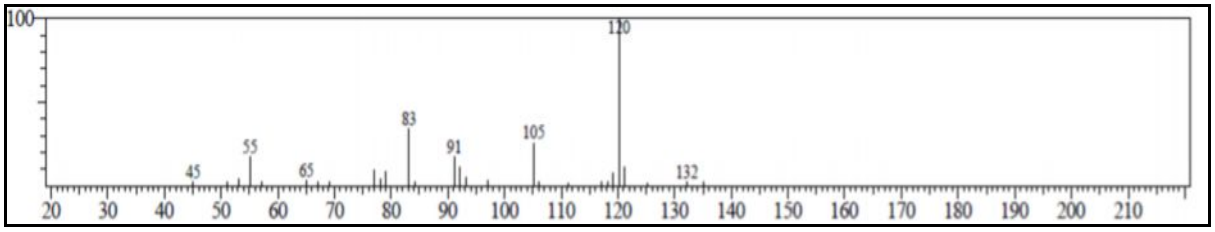

Figure 6. Spectrum of unknown compounds (of GC-MS) Comparative spectrum is as follows:

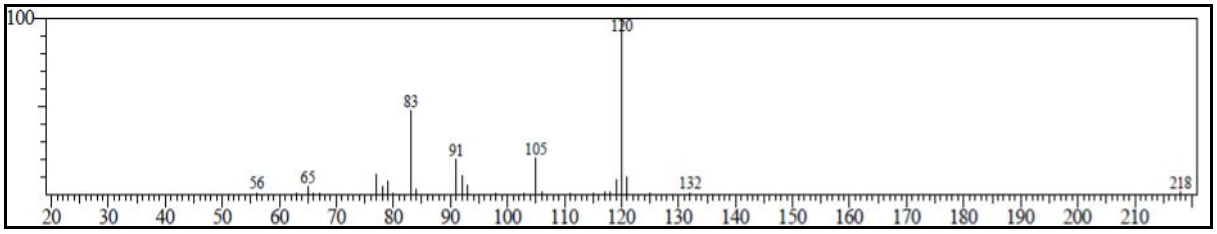

Figure 7 . ALPHA.-TUMERONE

Compound 4.

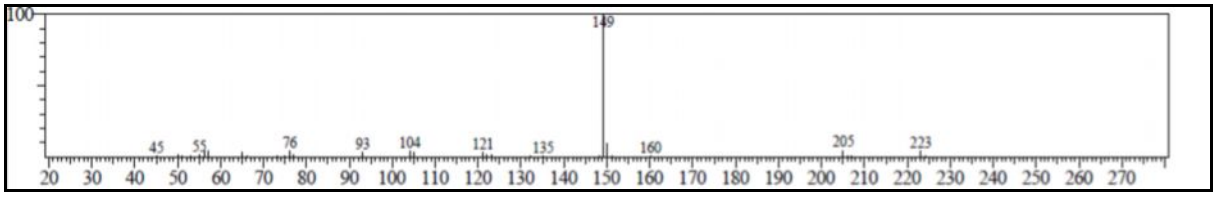

Figure 8. Spectrum of unknown compounds (of GC-MS)Comparative spectrum is as follows:

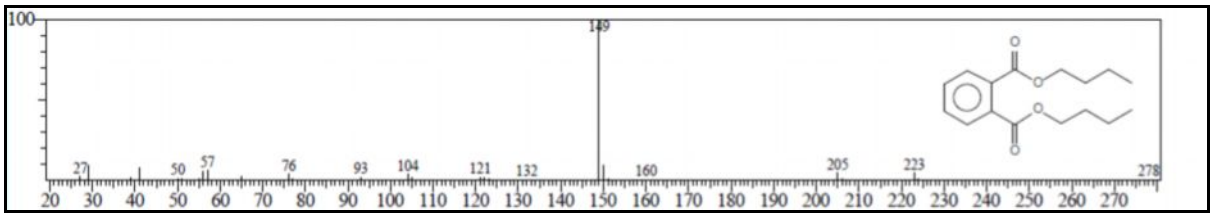

Figure 9. Dibutyl phthalate

Conclusion

Maceration results obtained:

1. Hexan Extract from the leaves of Morus alba .as much as 79.33 g
2. Ethyl acetate extract from leaves of Morus alba as much as 54.31 g
3. Phytochemical test results, the extract of ethyl acetate contains secondary metabolites: terpenoids and steroids,

Testing of anti-obesity activity of hexane and ethyl acetate extract is known, that the extract of hexane and ethyl acetate have anti-obesity activity, but the ethyl acetate extract were more active, so extract of ethyl acetate was fractionated and obtained.

4 groups of fractions, namely: A (32-42): 8:22 g; B (44-59): 1.34 g; C (61-68): 0.64 g, and D (71-84): 1.26 g.

Test antiobesity four groups of fractions, and her comparators, which was tested in five days, showed that:

1. Group of fracton A can reduce the average weight of the mice as -1.098 g.
2. Group of fraction B can reduce the average weight of the mice as -1.025 g
3. Group of fraction C can reduce the average weight of mice as much as -0.83 g
4. Group of fraction D raise the average body weight of mice as much as 0.92 g
5. Compounds Xenical (positive control) can reduce the average weight of mice as -1.098 g
6. Negative control (without treatment) raise the average body weight of mice as much as 0.85 g

Characterization of the ethyl acetate extract by GC-MS, containing:

Ar-tumerone (**1**); Tumerone (**2**); Alpha-Tumerone (**3**); Dibutyl phthalate **(4**); Cholesterol 3-O-[(2-acetoxy) ethyl (**5**)

Acknowledgments

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