CHARACTERIZATION OF COMPOUNDS FROM EXTRACT OF LEAF CLOROFORM *Melochia umbellata (Houtt.) Stapf var. Degrabrata K.* AND TEST ANTIHIPERGLICEMIC ACTIVITIES

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Abstrak. Penelitian karakterisasi senyawa dari ekstrak kloroform daun *M. umbellata (Houtt.) stapf var. degrabrata K.* dan uji aktivitas antihiperglikemik telah dilakukan. Penelitian ini bertujuan untuk mengisolasi dan mengkarakterisasi senyawa dari ekstrak kloroform serta menentukan konsentrasi optimum dari ekstrak kloroform dalam menurunkan kadar glukosa darah. Isolasi senyawa dari ekstrak kloroform dilakukan dengan cara maserasi, fraksinasi dan pemurnian. Berdasarkan hasil analisis spektroskopi IR dan NMR, senyawa yang berhasil diisolasi dari penelitian ini adalah β-sitosterol dan senyawa asam lemak yang terdiri dari 25 atom karbon. Pengujian toleransi kadar glukosa, konsentrasi 6% memperlihatkan penurunan kadar glukosa yang hampir setara dengan obat glibenklamid yang dijadikan pembanding.

Kata kunci: β-sitosterol, Uji toleransi kadar glukosa, ekstrak kloroform, dan Melochia umbellata

Abstract. A research on the characterization of the chloroform extract of leaves of *M. umbellata (Houtt.) stapf var. degrabrata K.* and activity test as antihyperglycemic has been done. This study aimed to isolate and characterize compounds from chloroform extract and determine the optimum concentration of chloroform extract in reducing blood glucose levels. Isolation compound from chloroform extract has been done by maceration, fractionation and purification. Based on the data of IR and NMR spectroscopic, compounds isolated from this research were β-sitosterol and fatty acid compound consisting of 25 carbon atoms. The glucose tolerance test, concentration of 6% showed a decrease in glucose levels almost equivalent with drug glibenclamide as comparator.

Keywords: β-sitosterol, Glucose tolerance test, chloroform extract, and *Melochia umbellate*

INTRODUCTION

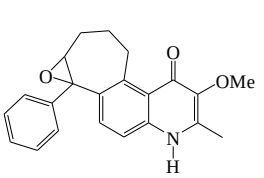
Exploration of plants as a source of medicinal ingredients has been carried out. Many plants have properties as traditional medicines, one of which is paliasa. Paliasa is one of the plants of the Malvaceae family that is used by the community, especially in South Sulawesi as a drug that can treat liver disease, hypertension, diabetes, high cholesterol and hepatitis by drinking boiled water from its leaves (Raflizar et al., 2006).

The name Paliasa is known in two different plant species namely *Kleinhovia hospita L.* and *Melochia umbellata (Houtt.) Stapf. Melochia umbellata (Houtt.) Stapf* consists of two varieties, namely *Melochia umbellata (Houtt.) Staple var. degrabrata K.* and *Melochia umbellata (Houtt.) staple*

var. visenia. As for the three types of paliasa, *Melochia umbellata (Houtt.)* Extract is *stapled var. degrabrata K.* is more toxic than other types of paliasa. This is evident from the results of research conducted by Lalo and Tayeb (2003) on the methanol extract of the three plant species at concentrations of 10% and 15% from *M. umbellata (Houtt.) Staple var. degrabarata K.* showed significant improvement in liver function. Likewise with the toxicity of *Artemia salina larvae*, the toxicity of methanol extract from the leaves of *Melochia umbellata (Houtt.) Staple var. degrabata K.* shows the highest. Bioactivity screening conducted by Nuvita (2006) from methanol extract of free radicals and BHT as a control also showed that the leaves of *Melochia umbellata (Houtt.) Staple var. degrabrata K.* has a higher antioxidant effect than other paliasa plants.

Based on the literature search, the chemical content found in the plants *Melochia umbellata (Houtt.)* Is *staple var. degrabrata K.* are essential oils, triterpenoids, alkaloids and flavonoids (Heyne, 1987); and saponin and interquinone compounds. The last two compounds can prevent inflammation of the liver in mice (Lalo and Tayeb, 2003).

Research on paliasa plants has been carried out. Many secondary metabolites have been isolated from this plant, both from extracts with polar, non-polar, and semi-polar solvents such as chloroform. Two quinolone alkaloid compounds were isolated from the chloroform extract of *Melochia umbellata (Houtt.) Staple var. degrabrata K.* namely 7.8-epoxy melochinon (3) (Erwin, 2010) and 9,10-epoxy melochinon (4) (Ridhay, 2012). Compound 4 is very active against P-388 leukemia cancer cells with lC50 = 0.83 µg / mL. The steroid secondary metabolite compounds were also isolated from the chloroform extract of *Kleinhovia hospita Linn.* (Soekamto, 2008) while for the terpenoid group, the 3-hydroxy-12-oleanan-28-oat compound (5) was also isolated from chloroform extract *K. hospita Linn.* (Purwaningsih, 2010).



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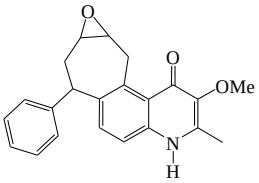

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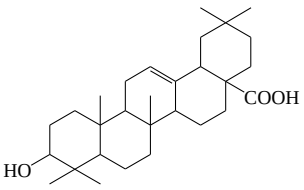

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Further studies of the bioactive compounds contained in the chloroform extract of the plant *Melochia umbellata (Houtt.) Staple var. degrabata K.* has been done. Although there have been many scientific reports about the ability of this plant to treat liver disease, research has not been done in relation to the treatment of diabetes. Therefore, this report about the plant *Melochia umbellata (Houtt.) Staples var. degrabata K.* which can reduce blood glucose levels.

MATERIAL AND METHODS

Materials

For extraction, fractionation and recrystallization used p.a and technical quality solvents which have been redestylated such as chloroform, methanol,

n-hexane, ethyl acetate and acetone. In the chromatography process used silica gel Catalog brand 7730 for vacuum column chromatography (KKV), silica gel Catalog brand 7734 for press column chromatography (KKT), silica gel Brand catalog 7733 for gravity column chromatography (KKG), and for thin layer chromatography (TLC) carried out with a silica gel-coated aluminum plate. Brand of brand 60 F254 0.25 mm. The stain-appearing solution used a solution of cerium sulphate ($Ce(SO_4)_2$) 2% in 2 N sulfuric acid and for glucose tolerance test (diabetes) used aquades, 9% glucose solution, Na-CMC solution and glibenclamide.

Tools

The tools used in this study were gram scales (O'hauss), scissors, oral spools, destillators, rotary evaporators, UV lights, tools for thin layer chromatography, vacuum column chromatography, compressive column chromatography, gravity column chromatography, and tools glassware commonly used in laboratories. For the measurement of melting point, it is done by using Fisher's tools John. While for the analysis of IR spectrometers with variants of FTIR 8501 Shimadzu, JEOL JMN A 5000 that works at 500.0 MHz for the 1H-NMR spectrum and 125.65 MHz for the 13C-NMR spectrum. NESCO® Glucometers are used for measuring blood glucose.

The Procedures

1. Plant Material Collection

Plant leaves Melochia umbellata (Houtt.) Staple var. degrabata K. was collected in April 2012 taken at the UNHAS Tamalanrea Campus, Jl. Perintis Kemerdekaan Km. 10, Makassar, South Sulawesi. This plant was identified by Herbarium Bogoriense, Biology Research and Development Center, LIPI Bogor with specimen number BO-1912171.

2. Extraction and Isolation

As much as 5 kg of dried powder from *Melochia umbellata (Houtt.) staple var. degrabata K.* was macerated with methanol for 1x24 hours 4 times. The maserate obtained is then concentrated and the crude extract obtained is then weighed to determine its total weight. Then fractionated by liquid-liquid extraction with n-hexane and chloroform. The chloroform extract obtained was evaporated to dryness then weighed and then fractionated through vacuum, press and gravity column chromatography using the appropriate eluent. Furthermore, the obtained isolates were purified by recromatography, crystallization and recrystallization. Structure determination is carried out based on IR and 1H and 13C-NMR spectrum data.

3. Glucose Tolerance Test

The chloroform fraction obtained was tested for its toxicity against adult male mice (Mus musculus). These experimental mice have been treated previously and have been given diabetes triggers. The drug preparation is varied in concentration. The data obtained is then recorded for further analysis.

RESULTS AND DISCUSSION

In the study of isolation of secondary metabolites from chloroform extract from the leaves of *Melochia*

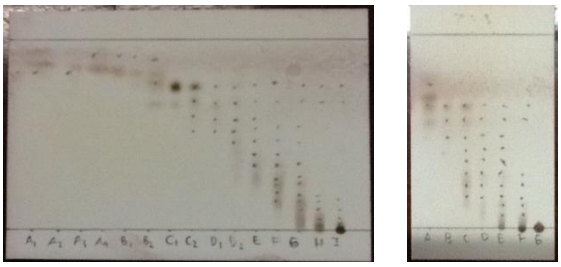
umbellata (Houtt.) Staple var. degrabata K. Two compounds were obtained. The two structures are determined based on IR and NMR spectroscopic data, namely compound 1 which is thought to be an β-sitosterol compound and compound 2 is thought to be a fatty acid compound consisting of 25 carbon atoms.

Extraction

Melochia umbellata (Houtt.) Leaf powder staples var. degrabata K. dried which was macerated with 1x24 hour methanol for 4 times to produce 39.1 g of dark green methanol extract. Then, the methanol extract was partitioned by liquid-liquid extraction with n-hexane, chloroform and ethyl acetate which produced n-hexane extract, chloroform extract and ethyl acetate extract. Then isolation and purification was carried out in the chloroform extract.

Isolation and Purification

Chloroform extract as much as 15.99 g was fractionated through vacuum column chromatography with elu-hexane, n-hexane: ethyl acetate, ethyl acetate, acetone and methanol which increased its polarity. The fractionation process begins with the search for eluents which show stains on the Rf value of 0.3 in the chromatogram analysis with TLC because the organic compounds are well separated at the value of the Rf. Stains with an Rf value of 0.3 are obtained from the ratio of eluent [n-hexan: ethyl acetate = 9: 1]. The fractionation results obtained were 15 fractions and combined into 7 main fractions (A-F fraction). Combining fractions based on the chromatogram of the TLC analysis results that have a similar stain profile (Figure 1). Fraction B weighing 244.3 mg was fractionated further by compressive column chromatography with eluent n-hexane, n-hexane: ethyl acetate, ethyl acetate and methanol. Fractionation produced 42 fractions which were combined into 8 main fractions. White crystals weighing 10.9 mg were formed in the B4 fraction. The purity test through analysis with TLC uses three types of eluent systems, each of which shows 1 stain that appears after being heated as shown in Figure 2.



(a) (b)

Figure 1. Chromatogram from the KLT analysis of chloroform extract, (a) results of KKV fractionation; (b) combined fractionation.

Furthermore, purity test through analysis with 2-dimensional TLC with eluent [n-hexane (7): ethyl acetate (3)] also shows one stain that appears after being heated but does not glow under the UV lamp short wave and long wave as shown in Figure 3 which indicates that the crystal is a pure isolate expressed as a compound 1. Test of the class of this compound shows a class of purplish purple steroids after addition of sulfuric acid and acetic anhydride.

(a) (b) (c)

Figure 2. Chromatogram analysis of TLC compound 1, (a) [n-hexane (9): ethyl acetate (1)]; (b) [n-hexane (8.5): $CHCl_3$ (1): methanol (0.5)]; (c) [Acetone (2): $CHCl_3$ (8)].

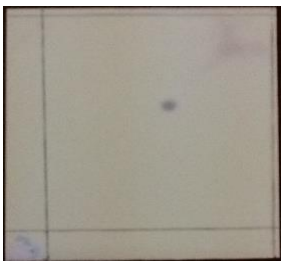


Figure 3. Chromatogram analysis of TLC 2 dimensional compound 1 after being heated.

A fraction weighing 596 mg was further fractionated by compressive column chromatography with n-hexane eluent: ethyl acetate, ethyl acetate, acetone and methanol to produce 28 main fractions as shown in Figure 4.

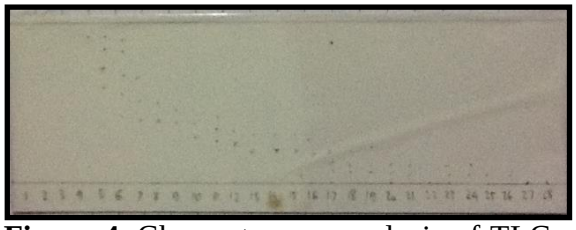
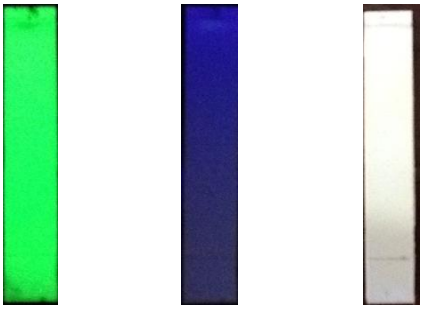


Figure 4. Chromatogram analysis of TLC fraction A, [n-hexane (9): ethyl acetate (1)].

Fraction 4 is then followed by recrystallization with hot acetone. The results of the A4 fraction recrystallization were then analyzed by TLC with ethyl acetate (2): n-hexane (8) and showed a single stain which was also supported by the yield of a melting point of 67-69 ^oC (Figure 5).



(a) (b) (c)

Figure 5. Chromatogram analysis of TLC compound 2, (a) UV short wave; (b) UV long wave; (c) after being heated.

Compound 1

Compound 1 which is in the form of white powder weighing 10.9 mg shows positive steroids by testing the Liebermann-Burchard reagent. This compound does not glow under UV lights which indicate that this compound does not have a conjugated double bond. This is in accordance with IR spectrum data which does not show the presence of absorption bands in the area of 3100-3000 cm^{-1} (Lambert et al., 1998) as wavelengths for aromatic groups.

IR spectrum data only shows the presence of an aliphatic CH group in the area absorption band 2956, 2926 and 2852 cm^{-1} which is supported by the presence of absorption in the regions 1462 and 1377 cm^{-1} for CH_2 and CH_3. The absorption band at wave number 3444 cm^{-1} indicates the presence of an O-H group supported by

absorption peaks at 1058 cm^{-1} for stretching vibrations of C-O. In addition there is also absorption at 1645 cm^{-1} for C = C which indicates the presence of olefin groups.

Table 1. spectrum IR of Compound 1

Functional group	Wavenumber (cm^{-1})	
	Compound 1	β-sitosterol (Sari, 2011)
O-H	3444	3452
C-O	1058	1049
CH aliphatic	2956 2926 2852	2954 2935 2891 2868 2850
CH_2	1462	1465
CH_3	1377	1381
C=C	1645	1627

The wave number in the IR spectrum of compound 1 does not show a significant difference with the IR spectrum of standard β-sitosterol compounds so that it can be concluded that this compound 1 is an β-sitosterol compound.

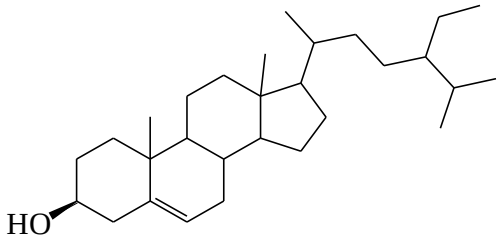


Figure 6. Structure of Compound 1 (β-sitosterol)

Compound 2

Compound 2 is in the form of white powder weighing 29.2 mg and melting point 67 - 69°C. IR spectroscopy showed absorption bands at wavelengths of 3448 cm-1 which showed the presence of O-H groups supported by C-O bending at wavelengths of 1172 cm cm^{-1}. The area of 2918 cm cm^{-1} and 2848 cm cm^{-1} indicated the presence of an aliphatic C-H group supported by methylene (CH_2) and methyl (CH_3) bending at wavelengths of 1467 cm cm^{-1} and 1379 cm^{-1}. Uptake in the area of 1636 cm^{-1}indicates the presence of olefins (C = C). IR compound 2 data can be seen in the following table.

Table 2. Spectrum IR of Compound 2

Functional group	Wavenumber (cm^{-1})
O-H	3448
C-O	1172
CH alifatik	2918, 2848
CH_2	1467
CH_3	1379
C=C	1636

The 1H-NMR spectroscopic data summarized in Table 3 shows 5 peaks. The first peak with a 0.88 ppm triplet-shaped chemical shift is a CH3 group that interacts with carbon that binds 2 protons (CH2). Chemical shift 1.29 ppm with multiplet multiplicity with very high intensity shows the presence of several CH2 groups in these wave numbers that have the same environmental conditions so that this second peak shows a very high peak. The third peak with a chemical shift of 1.61 ppm is in the form of a multiplet and the fourth peak with a chemical shift of 2.29 ppm is a triplet which interacts with carbon that binds oxygen. The fifth peak which is the peak with a chemical shift of 4.05 ppm which has triplet multiplicity is far from TMS because this peak is a group that binds oxygen.

Data on the 13C-NMR spectrum of compound 2 (Table 3) show 9 carbon signals consisting of methyl groups with chemical shifts of 14.31 ppm and 5 methylene in chemical shifts of 22.89; 25; 24; 26.14; 28.86 and 29.85 ppm, and 2 methyn groups in chemical shear 32.12 and 34.63 ppm and also carbon which binds to OH groups in chemical shifts of 64.59 ppm.

Table 3. ^{1}H and ^{13}C-NMR Data of Compound 2

$\delta\ ^{1}H$	$\delta\ ^{13}C$
0,88 (2H, t)	14,31
1,29 (2H, m)	22,89
1,61 (2H, m)	25,24
2,29 (2H, t)	26,14
4,05 (1H, t)	28,86
-	29,85
-	32,12
-	34,63
-	64,59

Bioactivity Test

The effect of giving CHCl3 extract leaves *Melochia umbellata (Houtt.) Staple var. degrabata K.* In mice for 3 hours showed a decrease in blood glucose levels (Table 4).

Table 4. Average blood glucose levels in mice due to leaf chloroform extract of *M. umbellata (Houtt.) Stapf var. degrabrata K.* with control and comparison

Treatment	**Glucose level after induction (mg/dl)**	**Glucose Levels Every Hour After Provision of Test Preparation Treatment (mg/dl)**			***Decrease Rate Glucose Level, K (mg/dl. jam)***
		1	**2**	**3**	
Na-CMC 1 % b/v	202,00	132,00	141,00	101,67	29,20
Glibenklamid 0,0195 mg/ml	237,00	137,00	90,67	73,67	53,63
Extract of Chloroform *M. umbellata* 2% b/v	233,67	136,33	129,67	108,67	38,17
Extract of Chloroform *M. umbellata* 4% b/v	248,67	172,67	125,67	105,00	47,80
Extract of Chloroform *M. umbellata* 6% b/v	258,33	129,67	119,67	94,67	50,10

Data from Table 4 shows a diagram of the decrease in blood glucose levels of mice as an effect of giving chloroform extract from *M. umbellata (Houtt.) Stapf var. degrabrata K.* which is accompanied by negative controls and comparators as shown in Figure 7 below.

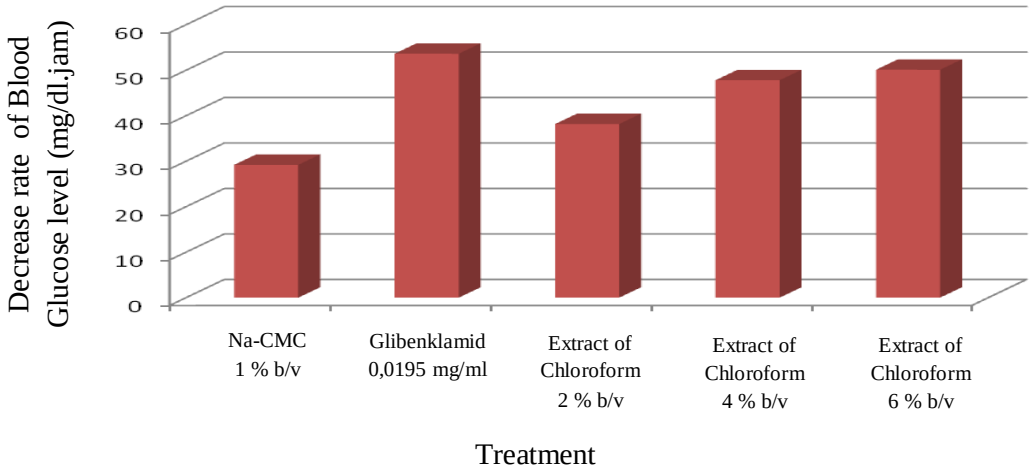


Figure 7. Profile of the decrease in blood glucose levels of mice due to the administration of *M. umbellata (Houtt.) Stapf var degrabrata K.* Chloroform Leaf Extract. with comparison of glibenclamide and negative control of Na-CMC 1% b / v.

Hyperglycemia is defined as fasting glucose levels higher than 110 mg/dL. Hyperglycemia arises because the absorption of glucose into cells is inhibited and its metabolism is disturbed while the condition of Diabetes Mellitus, diabetes or diabetes is a chronic disorder that is specifically related to glucose metabolism in the body, but metabolism of fat and protein is also disturbed.

This chloroform extract test was carried out to prove the pharmacological effect of plant leaves *Melochia umbellata (Houtt.) Staple var. degrabrata K.* as a drug that can treat diabetes and determine the optimum dose. This study used chloroform extract from these plants with concentrations of 2%, 4% and 6% b / v. 1% b / v Na-CMC suspension was used as a negative control and glibenklamide 0.0195 mg / ml was used as a positive control.

The antihyperglycemic effect was determined using the glucose tolerance method and the treatment conditions were kept uniform with the aim of reducing factors that might influence the results of the experiment.

The animals used in this study were male sex mice (Mus musculus) in healthy condition. Female mice are not used because the hormonal system is unstable compared to male mice. Nevertheless the biological variation factor of the test animal cannot be eliminated so that it can influence the results of the study.

Before treatment, mice were fasted to avoid the influence of food when measuring blood glucose levels and to increase the rate of absorption of drugs.

Measurement of blood glucose levels in mice is carried out for 3 hours with an interval of 1 hour. This is based on the literature which states that the

absorption of glucose in the body takes about 30-60 minutes and will decrease after 2-3 hours, so to see a clearer decrease in glucose levels, a period of 3 hours after the test preparation is given (Syahrul, 2010)

After measuring blood glucose levels for 3 hours, the diagram shows that the higher concentration of chloroform extract *Melochia umbellata (Houtt.) Staple var. degrabrata K.* The rate of decrease in blood glucose levels is also greater. The biggest influence is still shown by the comparison of chloroform extract of *Melochia umbellata (Houtt.) Staple var. degrabrata K.* at a concentration of 6% b/v.

CONCLUSION

Two compounds from chloroform extract from *Melochia umbellata (Houtt.) staple var. degrabrata K.* has been isolated. Compound 1 is thought to be a fatty acid compound that has 25 carbon atoms.

Chloroform extract from plant leaves *Melochia umbellata (Houtt.) Stapled var. degrabrata K.* can reduce blood glucose levels, this is in line with the use of this plant ethnobotany..

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