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3,6-dimethyl ester-α-mangostin Compound Modified from Isolate α-mangostin *Garcinia Mangostana* Linn

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***Abstract*—Isolate α-mangostin (1) is a phenolic compound derived from oxygenated and prenylated xanthones and major compounds obtained from the fruit peel of G. mangostana Linn., Which is stated to have inhibitory activity against α-glucosidase enzymes, serves to determine the antidiabetic activity resulting in IC50 value of 29,92 µM, is of the nature moderate to positive control (acarbose) with an IC50 value of 4.55 µM. A modified compound of α-mangostin (1) with acetic anhydride obtained by 3,6-di-methyl ester-α-mangostin (2) derivative showed the inhibitory value of α-glucosidase (IC50 13,89 µM), this value is better than the activity inhibition of α-mangostin (1), but not as active as the positive control value of the acarbose compound. The separation process to obtain α-mangostin isolates from the fruit peel of G. mangostana Linn was obtained by maceration method with ethyl acetate solvent, followed by refraction using a vacuum liquid chromatography (KCV) method over silica gel (Merck 60 G) and eluted using eluent (n-hexane: ethyl acetate) with increasing polarity, to produce as much pure crystal (21.66 g), yield (24%). While the structural characterization of the two compounds was carried out using UV-Vis, IR, HRESIMS, 1H-NMR and 13C-NMR spectroscopic methods, the antidiabetic testing was carried out using the α-glucosidase enzyme inhibition method.**

***Keywords*—*Garcinia mangostana*, α-mangostin, 3,6-dimethyl ester-α-mangostin, α-glucosidase inhibition.**

I. INTRODUCTION

DIABETES mellitus DM is a degenerative disease caused by chronic metabolic disorders characterized by excess glucose in the blood. This disease is caused by the body that cannot produce enough insulin (DM type I) or the body cannot use insulin effectively (DM type II). Diabetes mellitus (DM) has an increased incidence every year. Indonesia is ranked sixth in the world after China, India, the United States, Brazil and Mexico with the number of people with DM around 10.3 million people aged 20-79 years and this continues to increase [1]. Basic Health Research also reports a significant increase in diabetes, from 6.9% in 2013 to 8.5% in 2018 [2]. This is an important concern in the world of health.

One of the therapeutic options in the treatment of type II DM is α-glucosidase inhibitors. α-glucosidase is an enzyme in the small intestine. A-glucosidase inhibitors function to delay the absorption of complex carbohydrates so that it inhibits glucose production, by inhibiting the action of the α-glucosidase enzyme, the absorption of glucose will take place slowly so that glucose levels in the blood decrease. Antidiabetic activity can be done by testing on plants, this is because the ability of secondary metabolites they contain is able to restore pancreatic tissue function by increasing insulin production and inhibiting glucose absorption [3]. Secondary metabolites that have α-glucosidase inhibitor properties include α, β, and γ-mangostin compounds [4].

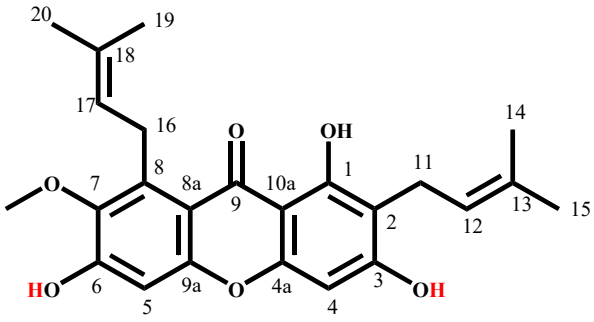

Figure 1. Structure of α-mangostin

Garcinia mangostana Linn. (mangosteen) is a species of the family Clusiaceae which has been reported as a source of prenylated and oxygenated xanthones [5]. Fifty xanthones were isolated from the skin of the *Garcinia mangostana* Linn fruit [6]. The main compound found in *G. mangostana* Linn. in the form of α-mangostin [7]. The extract of mangosteen rind ethyl acetate contains xanthones in the form of α-mangostin and γ-mangostin [8]. α-mangostin, one of the xanthones derivatives which is an active compound of the flavonoid group [9], has antidiabetic activity with an IC_{50} value of 53.3 µM against α-glucosidase inhibition [4], this value is still relatively moderate compared to acarbose (IC_{50} 4.55 µM) [10] which is often used as an oral drug for people with diabetes mellitus. Therefore, it is necessary to modify the α-mangostin compound through the acetylation reaction to produce the α-mangostin derivative compound as was done by previous researchers that the modification of the α-mangostin structure was reported to produce 3,6-di-methyl ester-α-mangostin which has better anticancer activity (ED_{50} 1 µM) than its parent compound (ED_{50} 4.1 µM) [11].

II. METHODOLOGY

A. Instrumen

The equipment used in this research are glassware, including: chamber, dropper, measuring pipette, capillary tube, vial bottle, filter paper, aluminum foil, airtight plastic (wrap), Merck TLC plates with silica gel 60 F254 (Merck KGaA 64271 , Darmstadt, Germany), preparative TLC plates, digital balance sheets (Fujitsu FS-AR210, Japan), maserators, rotary evaporators (Buchi R-210, Switzerland), Fisher John

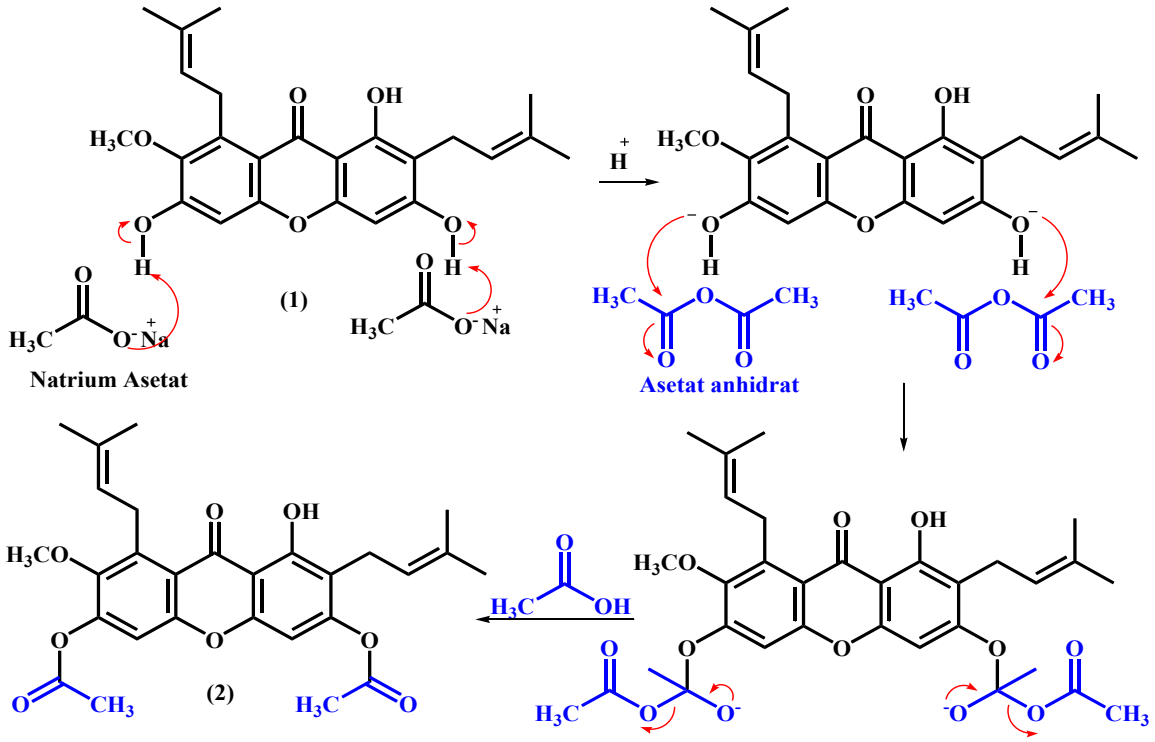

Figure 2. Proposed mechanism of the acetylation reaction.

Table 1.
Comparison of ^{13}C-NMR γ–mangostinand Compounds (2).

Posisi C	δ_C Compound **(1)** (aseton d_6, 100 MHz)	δ_C Compound **(2)** (aseton d_6, 100 MHz)	Posisi C	δ_C Compound **(1)** (aseton d_6, 100 MHz)	δ_C Compound **(2)** (aseton d_6, 100 MHz)
1	160.9	156.4	12	122.6	122.4
2	110.2	117	13	130.6	132.2
3	162.1	161.6	14	25.1	25.9
4	92.3	101.7	15	17.1	18
4a	155.4	151	16	26	27
5	102.8	111.9	17	123.9	123.9
6	158.7	154.9	18	137.3	132.5
7	154.9	148.1	19	25.1	25.9
8	143.6	139.3	20	17.5	18.4
8a	111.7	117.3	7-OCH_3	60.5	62.1
9	182	184.1	OAc		168.7
9a	101.9	107.6			20.9
10a	156.5	154.6	OAc		168.9
11	21.1	21			22.8

melting point Apparatus (Philip Haris, USA), equipment vacuum liquid chromatography (KCV), gravity column chromatography (KKG) equipment, UV lamps (λ = 254 and 366 nm), as well as UV-Vis Genesys 10S spectroscopy (Thermofisher, USA), FT-IR 8400S (Shimadzu, Japan), NMR DELTA2_JEOL ECS 400 MHz (JEOL, Japan), and HR-EI-MS (ThermoFinnigan, Bremen, Germany).

B. Material

The material used in this study was a sample of *G. mangostana* Linn fruit skin. from Bondowoso, solvent n-hexane (C_6H_{14}), dichloromethane (CH_2Cl_2), ethyl acetate ($CH_3COOC_2H_5$), and methanol (CH_3OH), as well as other materials namely Merck 60 G silica gel for KCV, silica gel 60 (70-230 mesh) for KKG, silica gel 60 GF_{254} as a stationary phase on the preparative TLC plate, reagent stain viewer of cerium sulfate ($Ce(SO_4)_2$) 1.5% in sulfuric acid (H_2SO_4) 2N, sliding reagents for UV tests namely NaOH, $AlCl_3$ and HCl, solvents for the NMR acetone-*d6* test, and KBr (potassium bromide) for the IR test.

The ingredients used for the modification of the α-mangostin compound are sodium acetate, anhydrous acetate, ice water, $MgSO_4$, petroleum ether, acetone, dichloromethane, and methanol.

The ingredients used for the α-glucosidase test are rat intestinal acetone powder (Sigma, 1630-10G), sucrose, phosphate buffer (Na_2HPO_4 and KH_2PO_4), glucose-kit liquor, acarbose, aquades, DMSO, HCl, and NaOH.

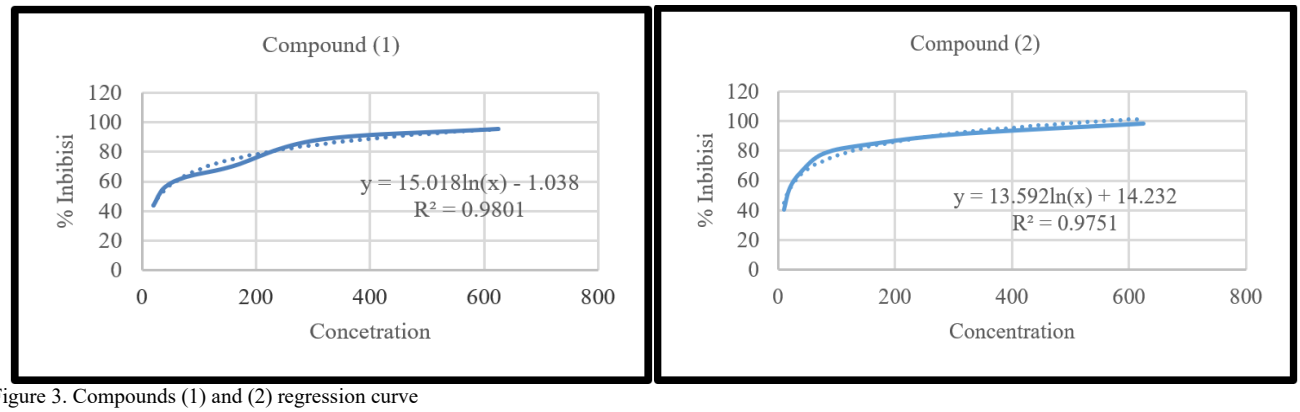

Figure 3. Compounds (1) and (2) regression curve

C. *Isolation of α-mangostin Compounds*

Isolation of α-mangostin compound was obtained from maceration of 8 kg of the skin sample of *Garcinia mangostana* fruit using ethyl acetate solvent. The extract obtained was evaporated with a rotary evaporator to obtain concentrated extract, then the concentrated extract was fractionated and refracted using a silica gel chromatography column. The pure compound obtained was characterized by a single stain profile on the chromatogram and then tested for purity with multi-eluent TLC, and melting point, then characterized by UV-Vis, FT-IR, HR-EI-MS, ^{1}H-NMR, and ^{13}C-NMR spectroscopy.

D. *Modification of α-mangostin Compounds*

Modification of the α-mangostin compound was carried out through the acetylation reaction with acetic anhydride reagents [11]. α-mangostin (41 mg; 0.1 mmol) is dissolved in anhydrous acetate (2.5 mL) then sodium acetate (10.3 mg; 0.125 mmol) is added. The reaction mixture is stirred with a stirrer at 60 ° C for 3-5 hours (monitored by TLC) followed by evaporation at low pressure. The residue obtained was partitioned with water (30 mL) and chloroform (15 mL), and the organic layer was separated, the solvent was evaporated at low pressure, and separated using chromatography eluent petroleum ether: acetone (8: 2) [12]. The acetylation product of the α-mangostin compound obtained was identified by the 13C-NMR spectroscopic method.

E. *Antidiabetic Test*

The antidiabetic test was performed using the α-glucosidase inhibition test method [3] using 10 µL test samples, 20 µL rat intestinal acetone powder, 30 µL 0.1 M phosphate buffer solution (pH 6.9), 80 µL glucose kit, and 20 µL sucrose substrate was incubated at 37 ° C for 10 minutes. Acarbose was used as a positive control, the absorbance was measured at λ = 520 nm using a UV-Vis spectrophotometer. Inhibitory activity is determined by the equation below

$$\text{Inhibition (\%)} = \frac{\text{Abs blank} - \text{Abs Sample}}{\text{Abs blank}} \text{ x } 100\% \qquad (1)$$

III. RESULT AND DISCUSSION

A. *Isolation of α-mangostin Compounds*

Isolation of α-mangostin compound was started by maceration process of 8 kg dry skin powder sample *Garcinia mangostana* Linn from Bondowoso East Java 3x24 hours using ethyl acetate @ 5L solvent to produce ethyl acetate extract, then ethyl acetate extract was evaporated with rotary evaporator getting 535.01 g (6.69%) concentrated ethyl acetate extract. Concentrated ethyl acetate (90 g) fractionated vacuum liquid chromatography method with silica gel 60 G (250 g) and gradient *n*-hexane-EtOAc (increasing polarity) eluent and monitored by TLC, to simplify the fraction stains on the TLC plate can be refracted until up to solids produced with a single stain profile on the chromatogram. Furthermore, solids with a single stain profile were tested for purity with multi-eluent TLC and melting point tests, and were characterized by UV-Vis, IR, HR-EI-MS, ^{1}H-NMR, and ^{13}C-NMR spectroscopy and α-mangostin (1) compounds were obtained. as in Figure 1 with a weight of 21.6643 g (24%).

B. *Modification of α-mangostin Compounds*

Structural modification can be carried out on isolated secondary metabolites to increase their activity [13]. The acetylation reaction is the reaction of entering an acetyl group (CH_3CO^+) into an organic molecular group such as (-OH and -NH_2). The most common reagent is acetic acid anhydride or acetyl chloride. The acetylation reaction to the OH group is important and is usually used in modification of organic compounds. Among the protective groups for OH, acetyl is a group commonly used because it is stable in acidic reactions and is easily removed by hydrolysis using alkalis. The acetylation reaction is the same as the esterification reaction, which is the reaction between alcohol and acid which produces ester and water [14].

Esterification reaction is the reaction between alcohols with carboxylic acids and their acid derivatives which will produce ester compounds. Making esters from phenols and carboxylic acids is very slow. Making esters from phenols can be well made from acid derivatives, such as acetic anhydride or acetyl chloride. This acid derivative is more reactive than carboxylic acid, so making esters from alcohols and acidic or acetyl chloride anhydrides can give better results [15].

The compound (1) which was isolated was modified by the acetylation and acetic anhydrous method as a reagent. α-mangostin (41 mg; 0.1 mmol) is dissolved in anhydrous acetate (2.5 mL) then sodium acetate (10.3 mg; 0.125 mmol) is added as a catalyst. The reaction mixture is stirred with a stirrer at 60°C for 3-5 hours (monitored by TLC) followed by evaporation at low pressure. The residues obtained were partitioned with water (30 mL) and chloroform (15 mL), and the organic layer was separated, the solvent was evaporated

at low pressure, and separated using a petroleum ether eluent chromatography: acetone (8: 2) produced acetylated mangostin (2) in the form of oily yellow orange as much as 13.5 mg (27%). The design of the reaction mechanism for the modification of α-mangostin compounds can be seen in the following Figure 2.

Modification of xanthone compounds by the acetylation method can occur in free hydroxy groups of xanthone frameworks [12]. The number of substituted free hydroxy groups by acetate can be determined by ^{13}C-NMR analysis, then the ^{13}C-NMR spectrum of compound (1) is compared with the ^{13}C-NMR spectrum of compound (2), increasing the amount of carbon in the ^{13}C-NMR spectrum of compounds (2) shows the number of groups acetate substituted free hydroxy.

The ^{13}C-NMR spectrum of compound (2) shows that there are four carbon signals added. 24 carbon in compound (1) to 28 carbon in compound (2), two methyl carbon signals namely in chemical shift (δ_C) 20.9 ppm and 22.8 ppm and 2 carbon ester signals ie at δ_C 168.7 ppm and 168.9 ppm. The 2 pairs of methyl and ester signals that appear are characteristic of 2 acetyl groups substituting 2 hydroxy groups on the α-mangostin compound to produce 3,6-di-methyl-ester-α-mangostin compounds (2). The following is a comparison of the ^{13}C-NMR spectrum of compounds (1) and (2).

C. Antidiabetic Test

Antidiabetic testing was carried out in vitro by the α-glucosidase inhibition method using the enzyme rat intestinal acetone powder (Sigma, 1630-10G), and sucrose as a substrate. In this test compounds that have α-glucosidase inhibitory activity will inhibit the action of α-glucosidase enzymes in the intestine so that it will slow down the breakdown of carbohydrates into glucose. The absorption intensity measurements of each compound were carried out at wavelength (λ) = 520 nm and the acarbose were positive controls. The compounds were tested to obtain an IC_{50} value or the magnitude of the concentration of compounds that can inhibit diabetes activity by 50%.

From the compound regression equation (1) and (2), IC_{50} values of compounds (1) and (2) can be calculated. Compound (1) obtained antidiabetic activity against α-glucosidase inhibition with an IC_{50} value of 29.92 μM while compound (2) was 13.89 μM. This shows that the substitution of the acetyl group against the free hydroxy compound α-mangostin can increase antidiabetic activity against α-glucosidase inhibition.

IV. CONCLUSIONS

Isolation of the skin of *Garcinia mangostana* Linn produced α-mangostin (1), while the modification of compound (1) by the acetylation method produces 3,6-di-methyl-ester-α-mangostin (2) derivatives. The modification results are known to be able to increase antidiabetic activity against α-glucosidase inhibition of α-mangostin (1) which has an IC_{50} value of 29.92 μM to become a compound of 3,6-di-methyl-ester-α-mangostin (2) which has a value of IC_{50} was 13.89 μM but IC_{50} compound (2) was still below IC_{50} acarbose as a positive control (4.55 μM).

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