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ANTI DIABETIC FLAVANONE COMPOUND FROM THE LEAVES OF *Artocarpus communis*

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ABSTRACT

The Flavanone compound with anti diabetic activity was isolated from ethyl acetate extract of Artocarpus communis leaves using column chromatography techniques. The structure of the flavanone compound was elucidated on the basic of spectroscopic evidence and comparison to published values. This compound, 8-geranyl-4,5,7-trihydroxyflavone, showed strong anti diabetic activity on α-glucosidase inhibition assay with IC_{50} 18.120 μg mL^{-1}.

Keywords: *Artocarpus communis, 8-geranyl-4,5,7-trihydroxyflavone, anti diabetic activity*

INTRODUCTION

Diabetes mellitus is the most serious chronic metabolic disorder and is characterized by high blood glucose levels [1-2]. At the present time, it is estimated that 150 million people worldwide have diabetes and that this will increase to 220 million by 2010 and 300 million by 2025. Globally, the percentage of type 2 diabetes (non insulin-dependent diabetes mellitus) is greater than 90% [3].

α-Glucosidase inhibitor has been used to treat type 2 diabetes mellitus. This drug does not increase insulin secretion. The antihyperlipidemic activity of α-glucosidase inhibitor comes from reversible inhibition on hydrolase, α-amilase pancreatic enzymes and intestine digestive enzymes, such as isomaltase, sucrase and maltase. These enzymes hydrolyze food carbohydrates to glucose and other monosaccharide. The α-glucosidase inhibitor inhibits the glucose absorption in the intestine acting as antihyperglicemia after carbohydrate intake [4-5].

As a number of anti-hyperglycemic agents have been found in plants, research into understanding the scientific basis for plant-based traditional medicine from various cultures has increased as scientists search for clues to discovering new therapeutic drugs for type 2 diabetes mellitus [6-8]. Traditional Indian medicines have long used plant and herbal extracts as anti-diabetic agent [9]. These plants are typically rich in phenolic compounds, which are known to interact with proteins and can inhibit enzymatic activity [10-11]. A number of medicinal plant and herbal extracts have been found to inhibit the enzymatic activity of α-glucosidase and α-amylase, and therein may have potential as dietary anti-diabetic agents to improve the control of postprandial hyperglycemia [12-15].

The genus *Artocarpus* (Moraceae), an exceptionally rich source of prenylated flavonoids, consists of approximately 50 species that are indigenous to the region of South East Asia, including Indonesia. Different compounds isolated from some species of *Artocarpus* have been shown to exhibit interesting biological properties [16-17]. Some of these compounds show interesting biological activities, such as cytotoxic [17], antimalarial activity [18], inhibition of tyrosinase and melanin biosynthesis [19-21]. Thus, in a continuation of our studies on the chemistry of Indonesian plants, the chemical constituents of *A. communis* have been investigated. In this paper, we report the isolation, structure elucidation and biological evaluation of prenylated flavonoid from ethyl acetate extract of the leaves of this species. The structure of this compound was elucidated on the basis of spectroscopic data including 2-D NMR. The isolated compound exhibited α-glucosidase activity using *in vitro* assay.

EXPERIMENTAL SECTION

Material

Sample of the leaves of *Artocarpus communis* was collected in March 2006, from plantation trees growing in Parung, Bogor, Indonesia. The plant was identified by staff at Biology Laboratory, Institute of Technology Bandung, West Java, Indonesia, and a voucher specimen has been deposited at Biology Laboratory.

Instruments

^{1}H- and ^{13}C-NMR spectra were recorded with JEOL JNM ECA-500 spectrometer, operating at 500 MHz (^{1}H-) and 125.76 MHz (^{13}C-), using TMS (Tetra Methyl Silane) as an internal standard. MS were obtained with Mariner Biospectrometry spectrometer

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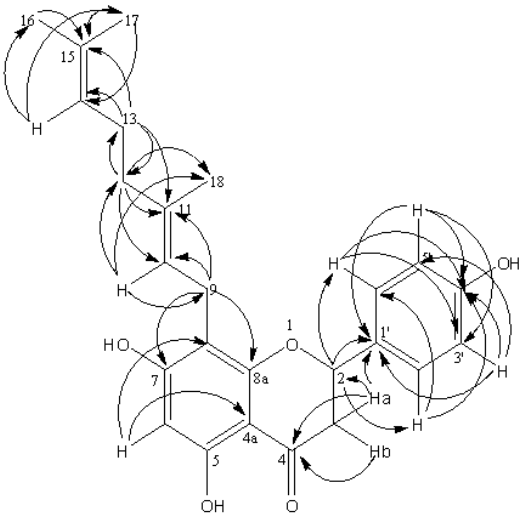

Figure 1. The HMBC correlation of compound AC-3-3

using ESI System (Electrospray Ionization) and positive ion mode. Column chromatography was carried out using Merck Silica gel 60 (70-230 mesh ASTM), and TLC (Thin Layer Chromatography) analysis on precoated Silica gel plates (Merck Kieselgel 60 F 254, 0.25 mm).

Procedure

Extraction, Isolation and Identification

The dried leaves (4.95 kg) of *A. communis* were extracted exhaustively using macerator with ethanol 70%. The ethanol extracts (250 g) were concentrated using vacuum rotary evaporator and then partitioned with hexane-water (1:4). Water extracts added with dichloromethane and then the residue was added with ethyl acetate. Ethyl acetate extract was fractionated by column chromatography on silica gel using gradient elution (hexane-ethyl acetate (8:2)), were resulted 70 fractions. Fractions 16-18, compound AC-3-3, were re-crystallized to give white crystal.

Compound AC-3-3 was identified using LC-MS and NMR (^{1}H-NMR, ^{13}C-NMR, DEPT 135, HMQC and HMBC) Spectrometer, to give flavanone compound, 8-geranyl-4,5,7-trihydroxyflavone.

Inhibition assay for α-glucosidase activity

α-Glucosidase inhibitory activity evaluation of the extracts was performed using established procedure [22]. α-Glucosidase enzyme solution was dissolved in phosphate-buffer solution (pH 7) containing 200 mg albumin serum. Before its application, 1 mL of the enzyme solution was diluted 25 times with the buffer solution. The reaction mixture consisting of 250 µL of 20 mM *p*-nitrophenyl α-D-glucopyranose as the substrate, 490 µL of 100 mM phosphate buffer (pH 7) and 10 µL of the extract dissolved in DMSO was prepared. The reaction mixture then was water-bath incubated at 37 °C for 5 min. The enzyme solution (250 µL) was added, and keeps the solution incubated for 15 min. The enzyme reaction was stopped by addition of 1000 µL, 200 mM sodium carbonate solution. The resulted *p*-nitrophenol from the reaction was measured at λ 400 nm. As positive control, the reaction of 1% of quercetin solution was measured. The commercial of α-glucosidase anti-diabetic drug, glucobay was available in the laboratory only in a form of sustain release tablet, therefore, quercetin is selected for positive control for *in vitro* evaluation. Samples concentrations for activity evaluation were 3.125, 6.25, 12.5, and 25 µg mL^{-1}, and 6.25, 12.5, and 25 µg mL^{-1} for quercetin.

RESULT AND DISCUSSION

The ethyl acetate extract from the leaves of *A. communis* was fractionated by column chromatography. A fraction containing the major components was purified by re crystallization to give 8-geranyl-4,5,7-trihydroxyflavone (AC-3-3).

AC-3-3, a colorless crystal, has a molecular formula of $C_{25}H_{28}O_5$ from LC-MS spectrum with $[M]^+$ ion at 409.0832.

The analysis of its NMR data, including HMQC and HMBC spectra, allowed for an unambiguous assignment of all proton and carbon signals. The ^{1}H-NMR data indicated the presence of three methyl groups at δ 1.55 (3H, *s*), 1.56 (3H, *s*), 1.61 (3H, *s*), three pairs of methylene protons at δ 1.91 (2H, *t*), 2.02 (2H, *t*), 3.18 (2H, *dd*), and two vinyl protons at δ 5.04 (1H, *t*) and 5.14 (1H, *t*) attributed to a geranyl group. In addition, proton signals suggested the existence of two aromatic rings, ring A and B. Ring A have proton aromatics symmetrically, at 6.81 (*d*, 8.56 Hz, 2 H) and 7.31 (*d*, 8.56 Hz, 2 H). At ring B, it has a sharp singlet at δ 5.93 indicated a chelated hydroxyl group. The ^{13}C-NMR spectrum indicated 25 carbons, including three methyl groups, a carbonyl group (δ 198.24), 8 sp^2 methine carbons, 4 methylene carbons, 3 methyl groups, and 9 quartenery carbons. These signals from ^{1}H- and ^{13}C-NMR suggested that this compound contained a geranyl substituent and flavanone group. The presences of the functional groups above were suggested by the long range coupling HMBC experiment in Fig. 1. The multiplicity of carbons were assigned by the DEPT-135 experiment and correlation of the chemical H and C shift for all protonated carbons was determined based on the HMQC spectrum as summarized in Table 1.

From 1D- & 2D- NMR spectrum, MS data, and comparison with previous spectral data [23-24], compound AC-3-3 was identified as flavanone group,

Table 1. ^{1}H- and ^{13}C-NMR spectral data of compound AC-3-3*

No	δ_H (∑H, multiplicity, *J* in Hz)	δ_C
2	5.3 (1H, *dd*)	80.37
3	Ha. 2.70 (1H, *dd*) Hb. 3.06 (1H, *dd*)	44.12
4		198.24
4a		103.45
5		163.24
6	5.93 (1H, *s*)	96.47
7		161.71
8		109.16
8a		166.16
9	3.18 (2H, *dd*)	22.48
10	5.14 (1H, *t*)	124.10
11		135.29
12	1.91 (2H, *t*)	40.97
13	2.02 (2H, *t*)	27.78
14	5.04 (1H, *t*)	125.58
15		132.09
16	1.55 (3H, *s*)	17.86
17	1.61 (3H, *s*)	25.98
18	1.56 (3H, *s*)	16.34
1’		131.48
2’, 6’	7.31 (2H, *d*, *J* 8.56 Hz)	129.04 (2C)
3’, 5’	6.81 (2H, *d*, *J* 8.56 Hz)	116.34 (2C)
4’		159.00

* Spectra recorded at 500 MHz for ^{1}H spectrum and 125 MHz for ^{13}C spectrum in CD_3OD. The values are in ppm and *J* values (Hz) in parentheses. Abbreviations for NMR signal are as follows: *s*= singlet, *d*= doublet, and *t*= triplet. Correlation of chemical shift H and C were assigned, based on the HMQC spectra.

8-geranyl-4,5,7-trihydroxyflavone ((E)-8-(3,7-dimethylocta-2,6-dienyl)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one) (Fig. 2).

The antidiabetic of this compound was evaluated according to the method previously described [23]. This compound exhibited significant activity in the α-glucosidase inhibitor with IC_{50} 18.120 µg mL^{-1}. This value has higher activity than quercetin as positive control (Table 2).

CONCLUSION

Based on LC-MS, ^{1}H-NMR and ^{13}C-NMR (1D & 2D) spectra, and compared with previous spectral data, compound AC-3-3 from ethyl acetate extract of *A. communis* leaves was identified as flavanone group, 8-geranyl-4,5,7-trihydroxyflavone ((E)-8-(3,7-dimethylocta-2,6-dienyl)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one). This compound showed significant activity in the α-glucosidase inhibitor with IC_{50} 18.120 µg mL^{-1}.

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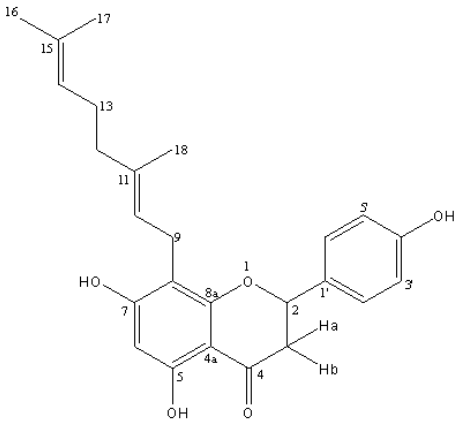

Figure 2. The molecular structure of compound AC-3-3

Table 2. The α-glucosidase inhibitory activity of compound AC-3-3 from ethyl acetate extract of *A. communis* leaves compared with quercetin

Sample	Concentration (µg mL^{-1})	Absorb value	% Inhibition*	IC_{50} (µgmL^{-1})**
AC-3-3	3.125	0.552	58.311	18.12
	6.25	0.539	39.410	
	12.5	0.452	27.748	
	25	0.311	26.005	
Quercetin	6.25	0.318	14.050	25
	12.5	0.273	26.220	
	25	0.180	51.35	

* % Inhibition: [(Concentration-absorb value)/Concentration] x 100
** IC_{50}: Concentration of inhibitor to inhibit 50% of its activity.

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