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Inhibitory of activity α-glucosidase from ethyl acetate and flavanal compound of the stem-bark of *Calophyllum mcrophyllum* Scheff

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Abstract. Most of Calophyllum genus have been researched and proven as medicinal plants. *Calophyllum macrophyllum* Scheff is an Indonesia original plant growing on Sumatera and we found in Kerinci mount. One flavanal has been isolated from ethyl acetate fraction from the stem-bark of *Calophyllum macrophyllum* Scheff. The compound is 5,7,2',5'- tetrahedrons flavan-3-ol, brown crystal with melting point > 300°C. The compound was extracted using maceration technique, then fractionated using chromatography and purified by crystallization using two solvents. Molecule structure was determined using physical data and spectroscopy methods including UV, IR and ^{1}H and ^{13}C-NMR. Ethyl acetate fraction and isolate were assayed for inhibitory activity on α-glycosidase enzyme. The potential inhibition effect is got each other with IC_{50} 26,198 µg/ml and 12,19 µg/ml.
Keywords: *C. macrophyllum* Scheff, Clusiaceae, Flavanal, inhibitory of α-glycosidase

Introduction

The genus *Calophyllum* of the Guttiferae family is a large group of tropical trees consisting of approximately 200 different species. The genus *Calophyllum* is primarily found in the Indo-Pacific region, particularly Malaysia (Crane, S et *al,* 2005). In Indonesia, this plant is commonly known as nyamplung (Suryanegara, 1994). and a number of medicinal and therapetic properties have been described to various parts of *Calophyllum* tree, including the treatments of rheumatism, varicose vein, hemorrhoids and chronic ulcers (Cottiglia et al 2004). The balsem from the bark of *C inophyllum* Alexandrian Laurel used as a cicatrisan, infusion or decoction of the leaves has been traditionally used as an eye remedy in Asian medicine (Iinuma M, 1993). Bioctivity of xanthon from Calophyllum as antihypoglycaemic, antiplatelet, antimicrobial, (Iinuma M, 1996), antiimflammatory, antifungal, inhibition of lipid peroxidase (Iinuma M, 1993), prenylcoumarin have activity as antitmor (Itoigawa M, 2001), class of coumarin (such as costatolide, soulatrolide, calanolide A) that the presence of a benzylic alcohol at C10 was important for anti-HIV activity (Kirk R, 1994). Polyiisoprenylated ketone, enervosanone from the stem bark of *C. enervosum* have activity as antimicrobial (Taher M, 2005)

Calophyllum species which are in cromene acid, most of these acids possess a phloroglucinol ring system, such as the isocordato-oblogic acid from the *n*-hexane extract of the stem bark of *C. cordato-oblongum* (Dharmaratne H.R.W et al 1999). Theses spesies are also particularly rich in coumarin derivates, calanolide F, soulattrolide and costatolide and other coumarin were isolated from the leaf, bark latex, and twig (Cao Shu-Geng).

C. macrophyllum Scheff is one of genus *Calophyllum*, but information chemical compounds and its bioactivity of this plant wasnot reported yet. According to the reference (Suryanegara, 1994). the large tree of *C. macrophyllum* Scheff up to 45 m tall, with bole up to 160 cm in diameter. It grows up to 800 m altitute. In secondary forests usually

present as a pre-disturbance remnant tree. Distribution in Southern Thailand, Peninsular Malaysia, Singapore, Sumatra and Kalimantan Islands. The wood is used for construction purposes. The fruit is edible.

Here we wish to report the isolation, structural elucidation, and bioactivity from ehtyl acetate extract of stem bark of *Calophyllum macrophyllum* Sheff. The specimen was extracted using maceration technique, then fractionated using chromatography and purified by crystallization using two solvents. Molecule structure was determined using physical data and spectroscopy methods including UV, IR and ^{1}H and ^{13}C-NMR. Ethyl acetate fraction and isolate were assayed for inhibitory activity on α-glucosidase enzyme.

Materials and Methods

General

Liquid chromatography : silica gel 60 (particle size 70-230 mesh and 230-400 mesh) and silica H. TLC silica gel precoated alluminium plates. (merck, silica gel *60 F_{254}*). Spot were visualized by UV (λ_{254max}) and 5% H_2SO_4. UV Spectra were recoerded on a Hitachi spectrophotometer in nm. IR spectra were measured on a Shimadzu Prestige 21 FTIR Spectrometer in KBr pellet ; ύ in cm^{-1}. NMR Spectra Jeol–JNM ECA 500 (500 ^{1}H and 125 ^{13}C) instruments using $CDOD_3$: unless otherwise state ∂ in ppm, *J* in Hz. LC-MS Mariner Bio Spectrometry. Melting point for isolated crystals were measured on a Fisher Scientific serial 903N0056 apparatus. And bioassayed for inhibitory activity on α-glycosidase enzyme.

Plant material

The stem bark of *C.macrophyllum* Scheff colected from the Kerinci mount forest on Mei 2009 and identified by Mr Ismail Rahman is a botanis. A voucher specimens was compared with the herbarium specimens at the Herbarium Research Centre for Botanic. Bogor, and deposited at the Herbarium Research Centre for Botanic in Bogor. Indonesian

Extraction and isolastion of plant materials

The bark of *C.macrophyllum* Scheff was dried at room temperature then at oven 50°C and powdered. The dried powdered 3.2 kg *of C. .macrophyllum* Scheff was successively and exhaustively extracted with ethanol 70%. Evaporation of solvent gave 477,5 g ethanol extract was then suspended in water and partitionated with hexane, ethyl acetate and n-butanol.

Ethyl acetate extrac was fractionated by flash column chromatography (silica gel Merck 800 gr) eluting with n-hexane, a gradient of ethyl acetat to 100% followed by ethyl acetate/MEOH (20:1, 10:1, 5:1, 1:1). Fraction with the same R_f valeus were combined. Fraction E-G from (100% Ethyl acetate then Ethyl acetate in MeOH 20:1, 10:1) were combined. Then was subjected to column chromatography on silica gel 230-400 mesh eluting with n-hexane-ethyl acetate-methanol with a gradient sytem. Isolate recristalized by using CH_2Cl_2 and methanol

Inhibition assay for α-glucosidase enzyme

The reaction mixture consisting 250μL 0f 20 mM p-nitrophenyl α-D-glucopyranoside (Sigma Chemical Co.), 495 μL phosphate buffer (pH 7.0) adding to flask contain 5 μL of sample dissolved in DMSO at various concentration (3.135-25 μg mL^{-1}). The reaction mixture was

pre-incubated for 5 min at 37^0C, the reaction was start by adding 250 µL α-glucosidase (0.075 unit) (EC 3.2.1.20 from Wako Pure Chemical Industry) incubation was continued from 30 min. The reaction stopped by adding 1 ml 0f 0.1 M Na_2CO_3. Activity of α-glucosidase was determined by measuring release of p-nitrophenol at 400 nm. Koji extract from *Aspegillus terreus* used positive control of α-glucosidase.

Result and Discussion

Ethanol extract was subjected to the water, were then partitionated by n-hexane, Ethyl acetat and n-buthanol. Then each other dried by rotary evaporator. The drying extract showed as below (Tabel 1)

Table 1. Partitioned result of samples

N0	Sample	Partitioned results		
		n-hexane	Ethyl acetate	n-butanol
1	*C.macrophylum* Scheff (3,2 kg)	5 g	53,9 g	47,8g

Isolate

We have succesfullly isolated flavanal from ethyl acetate fraction of *C.macrophyllum* Scheff as brown crystal. The molecular formula was determined as $C_{15}H_{14}O_6$, Mol weigh 290 by LC-MS. The UV spektrum showed typical absorption λ_{max} at 222, and 278 nm. Infra red specrum with KBr pellet showed absorption bands at υ_{max} 3496, 3174, 2989, 2931, 1440, and 1143 $cm^{-}1$.

Table 2. Chemical shift, HMQC, HMBC, and DEPT of isolate (ppm)

No.C	H-NMR	HMQC	HMBC (δ ppm)					DEPT
		δ ppm	1	2	3	4	5	
2	4,81,(*s*)	80	29,4	115,4	115,9	132,4		CH
3	4,18 (*m*)	67,6						CH
4a	2,73 (*dd*, 16,8 & 2,75)	29,4	67,6	80	100,2			CH_2
4b	2,86 (*dd*, 16,5 & 4,3)				100,2	157,5	157,7	
5	/	157,5						
6	5,91 (*d*, 1,8)	96,5	96	100,2	157,5	158,1		CH
7	/	158,1						
8	5,94 (*d*, 1,85)	96	96,5	100,2	157,7	158,1		CH
9	/	157,7						C-quer
10	/	100,2						C-quer
1'	/	132,4						C-quer
2'	/	145,9						C-quer
3'	6,76 (*d*, 8,5)	115,9	80	115,4	132,4	145,9		CH
4'	6,80 (*dd*, 8,25 & 1,6)	115,4		115,9	132,4	145,9	146	CH
5'	/	146						
6'	6,97 (*d*, 1,2)	119,5	80	145,9	146			CH

The band at 3496 cm^{-1} was assigment hydroxy. The band at 3174 cm^{-1} was assiggned to aromatic ring (C-H streching) and C=C bending at 1440 cm^{-1}. The band at 2989, 2931 cm^{-1} were assiggned to C-H aliphatic stretching. The band at 1143 cm^{-1} was assiggned to C-O.

The ^{1}H NMR spectrum showed four protons with two ring aromatics. Firts aromatic have two protons with metha chemical shift 5.91 (1H, *d, J* = 1.8 Hz) and 5.94 (1H, *d, J* = 1,85 Hz) (ring-A) and the second have three protons with metha and ortho chemical shift 6,76 (1H*, d*, *J* = 8.5); 6.80 (*dd*, *J* = 1.6 & 8.25); 6.97 (1H, *d, J* = 1.2) (ring-B). Two protons at 4.81 dishelding and 4.18 as methine respectively and two protons (methilene) with chemical shift 2.73 (1H, *dd, J* = 2.75 and 16.8 Hz) and 2.86 (1H, *dd,* 16.5 and 4.3 Hz) as germinal coupling. This data suggested two ring aromatic connected by 3 C. It could be make cyclization (ring-C) as Figure 1. If the units above combination, It can make structure as Figure 2. Analysis 2D by HMBC from table 2 would show as Figure 3.

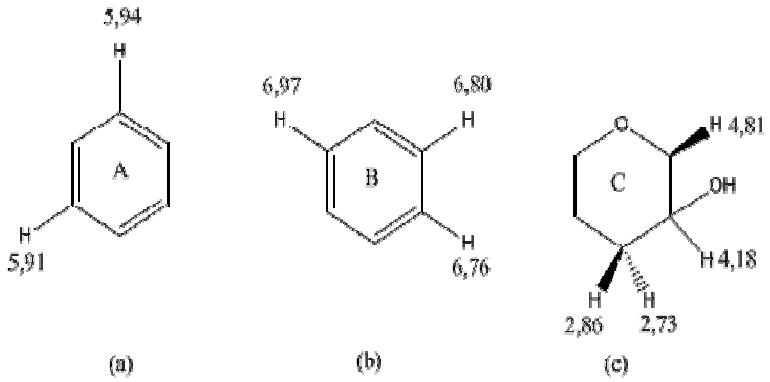

Figure 1.Two ring aromatic connected by 3 C make cyclization (ring-C)

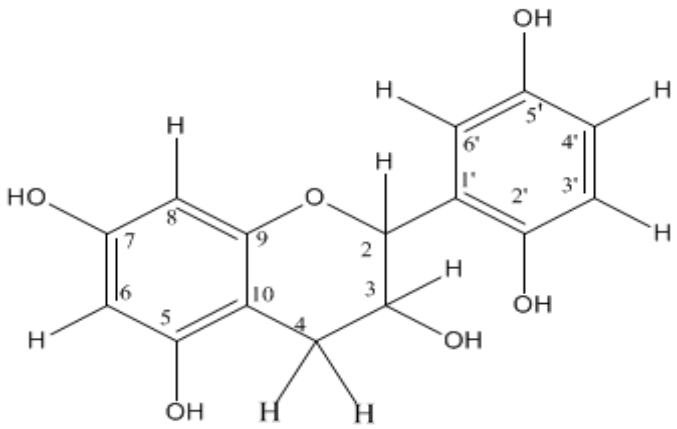

Figure 2. Structure of combination unit

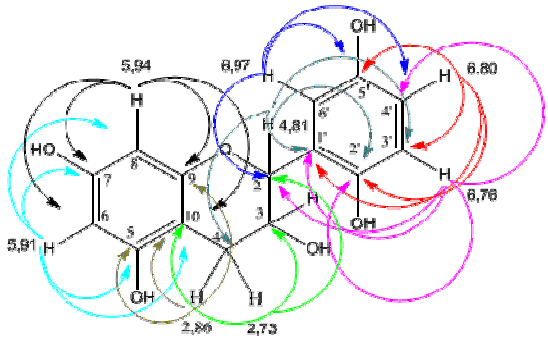

Figure 3. Analysis 2D by HMBC

Bioactivity

Screening of phytocemistry with Ciuley (1984) method showed that ethyl acetate extract of *C.macrophyllum Scheff* contain flavonoid compound. According Sutedja (2003) that flavonoid compounds had been potency as antidiabetes. In this investigation will be showed the potency ethyl acetate extract and isolate of *C.macrophyllum Scheff* as inhibitory agent on α-glucosidase enzyme. The result bioactivity showed in **Table 3**.

Table 3. Inhibitory of ethyl actate extract and isolate of *Calophyllum macrophyllum* Scheff in various concentration compare with koji as a control.

SAMPEL	ABS	KONS	IHH	A	B	R	IC_{50}	
Blanko	1.792							
Standar koji	0.135	25	92.46652	17.42	25.15	0.925	1.29	Active
	0.215	12.5	88.00223					
	0.586	6.25	67.29911					
	1.053	3.125	41.23884					
Ethyl acetate fraction	0.931	25	48.04688	12.3	1.439	0.999	26.19	Active
	1.241	12.5	30.74777					
	1.405	6.25	21.59598					
	1.5	3.125	16.29464					
Isolate	0.298	25	83.37054	15.08	2.863	0.959	12.19	Active
	0.727	12.5	59.4308					
	1.245	6.25	30.52455					
	1.411	3.125	21.26116					

The investigation use a koji extract as control from *Apergillus terreus* is an especially prolific producer of secondary metabolites has biological activities such as inhibitory of α-glucosidase and it has a most potential activity therefore examined the effect on postprandial blood glucose level after a meal in mice (Triana et *al*. 2007). From **Table 3**

showed that in lower concentration sample has a small inhibition but rise with increasing of the concentration.

Conclusion

In conclusions the fraction of ethyl acetate extract and isolate of *C.macrophyllum Scheff* showing potential activity α-glucosidase inhibitory on *in vitro* assay. The potential inhibition effect (IC_{50}) was 26.198 µg/ml and 12.19 µg/ml respectively.

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