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Research Article

ANTIOXIDANT ACTIVITY OF FLAVONOID FROM *ANREDERA CORDIFOLIA* (TEN) STEENIS LEAVES

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ABSTRACT

A flavonoid 8-Glucopyranosyl-4',5,7-trihydroxyflavone, have been isolated from the methanol extract of leaves of *Anredera cordifolia* (Basellaceae). The structures of these compounds were established based on spectroscopic evidence, including UV, IR, ^{1}HNMR dan^{13}CNMR spectra.Antioxidant activity test by using DPPH (1,1-diphenyl-2-picrylhydrazyl) method from the isolated compounds showed antioxidant DPPH radical scavenging their IC_{50} values were 68,07µg/mL.

Keywords: *Anrederacordifolia,* 8-Glucopyranosyl-4',5,7-trihydroxyflavone, DPPH

INTRODUCTION

Anredera is one species of the Basellaceae which empirically has many benefits in health, especially for treating various diseases. Familia Basellaceae have a variety of species such as *Anredera baselloides* (Kunth) Baill, *Anredera cordifolia* (Ten.) Steenis, *Anredera diffusa* (Moq.), *Anredera leptostachys* (Moq.)Steenis, *Anredera spicata*, *Anredera vesicaria*, *Anredera cumingii*, and others[1]. One species that is widely used by people in Indonesia are *Anredera cordifolia* locally known as"binahong". Potential as amedicinal plant because of the bioactive compounds from these plants. Screening of phytochemicals known to contain flavonoids, saponins, steroids/triterpenoids and coumarins[2]. Flavonoid class of compounds known to have diverse biological activities such as antioxidant . In this paper will be delivered the discovery of a flavonoid glycoside compound of ethylacetate extract of leaves binahong. The molecular structure of compounds was determined based onspectroscopic data UV, IR, ^{1}HNMR, ^{13}CNMR

MATERIALS AND METHODS

Plant material

The selected medicinal plant *Anredera cordifolia* shoots were collected from the Bogor Botanic Garden, and identified from the Department of Botany, Indonesia Institute for Science where a voucher specimen number has been deposited.

Extraction and isolation

The plant material were dried under shade and ground to a coarse powder (4 kg) was extracted exhaustively with methanol at room temperature. The combined extract was evaporated to dryness on a rotary evaporator. The dried methanolic extract further successively partitioned with *n*-hexane, ethyl acetate and finally with *n*-buthanol. The filtrates were concentrated dried under vacuum

The ethyl acetate extracts was fractionated by VLC (*Vacuum Liquid Chromatography*) using gradient elution with dichlormetane-isopropanol-methanol. The subfractions, were combined to give nine fractions. Furthermore, fraction 9 was isolated further using sephadex column chromatography eluted with methanol, obtained 250 fraction. Fractions which gave the same chromatographic pattern obtained seven fractions combined. Fraction 9-5 chromatographed again with sephadex column and eluted with methanol, obtained 300 fractions, obtained seven fractions combined. Fraction 9.5-4 was obtained yellow powder and after crystallization, compound (**1**) was obtained. It was elucidated by UV, IR, LC-MS and NMR spectroscopies.

Antioxidant activity assay

The DPPH assay was done according to the method of Brand-Williams et al. (1995) with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at 20°C until needed. The working solution was obtained by mixing10mL stock solution with 45mL methanol to obtain an absorbance of 1.170.02 units at 515 nm using the spectrophotometer. The extracts (150 mL) were allowed to react with 2850 mL of the DPPH solution for 24 h in the dark. Then the absorbance was taken at 515 nm. The standard curve was linear between 25 and 800 mMTrolox. Results are expressed in mM TE/g fresh mass. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve[3].

RESULTS ANALYSIS

From the extracted binahong leaves (*Anrederacordifolia* (Ten.) Steenis) with ethyl acetate flavonoid glycosides found in a compound. This compound is obtained through several stages of isolation include partitioning, fractionation and various chromatographic techniques. Compound (**1**) was obtained as a yellow powder, mp.249-250 ^{0}C , $C_{21}H_{20}O_{10}$ (m/z 432,06) $[M+H]^{-}$ .Spectrum showed UV absorption at λmaks273,0 nm(band II); 337,0 nm (band I) indicating the precence of flavonoid (Figure 1) , while the IR spectrum showed absorption bands (vmaks) are typical for hydroxyl 3640 cm^{-1},-carbonyl group 1649 cm^{-1}, and the aromatic rings of appeared at 1613, 1568, 1506 cm^{-1}.

The ^{1}H-NMR spectrum of (**1**) showed the presence of signals for sugar at δ_H3,26-4,70 , a hydrogen bonded of hydroxyl group with carbonyl group at δ_H13,16 (1H, 5-OH, s), aromatic signal at δ_H6,25 – 8,01, two *orto*-coupled aromatics proton at δ_H 6,88 and 8,01 (each 1H, *s*) and two singlets (δ_H 6,25 and 6,76). In its ^{13}C-NMR spectrum, the presence 21 carbon signals including conjugated carbonyl carbon at δ_C 182,01 a methylene hydroxide at δ_C 61,30, five signals CH in area sugar δ_C70,88 (C-2"); 70,55 (C-4"); 73,43 (C-1"); 78,70 (C-3"); 81,84 (C-5") and fifteen aromatic carbon was observed. From these data, this compound was regarded as a

glycoside flavonoida. Based on the long –range correlation between H-3 (δ_H6,76) and δ_c103,77 (C-10), δ_C 182,01 (C-4), δ_C163,85 (C-2), δ_C 121,59 (C-1'); H-6 (δ_H6,25) and δ_c 103,77 (C-10), δ_C 163,85 (C-2), δ_C 160,41 (C-7); H-2' (δ_H 8,01) and δ_C 161,25 (C-4'), δ_C 163,85 (C-2); H-3' (δ_H 6,88) and δ_C 121,59 (C-1'), δ_C 161,25 (C- 4'); H-5' (δ_H 6,88) and δ_C 121,59 (C-1'), δ_C 161,25 (C- 4'); H-6'(δ_H 8,01) and δ_C 161,25 (C- 4'), δ_C 163,85 (C-2); H-1" (δ_H 4,70) and δ_C 104,66 (C-8), δ_C 156,04 (C-9); H-2" (δ_H3,84) and δ_C73,43 (C-1"), δ_C78,70 (C-3") ; H-3" (δ_H 3,27) and δ_C 70,55 (C-4"); H-4"(δ_H 3,38) and δ_C 78,70 (C-3"); H-5" (3,26) and δ_C 70,55 (C-4") in its HMBC spectrum (Figure1, Table 1). Therefore, **(1)** determined as 8-Glucopyranosyl-4',5,7-trihydroxyflavone (Figure 2).

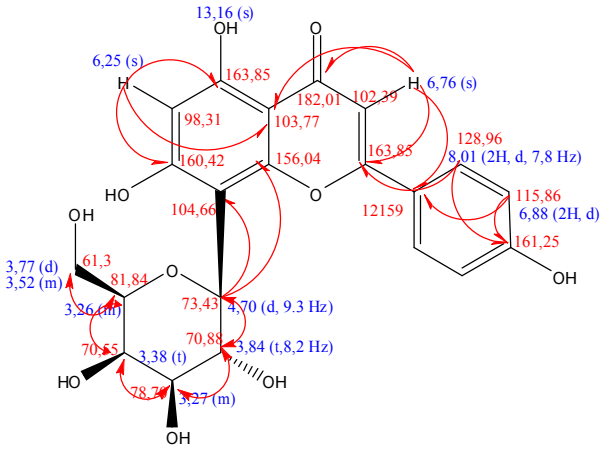

Fig.1. key long-range correlation of (1)

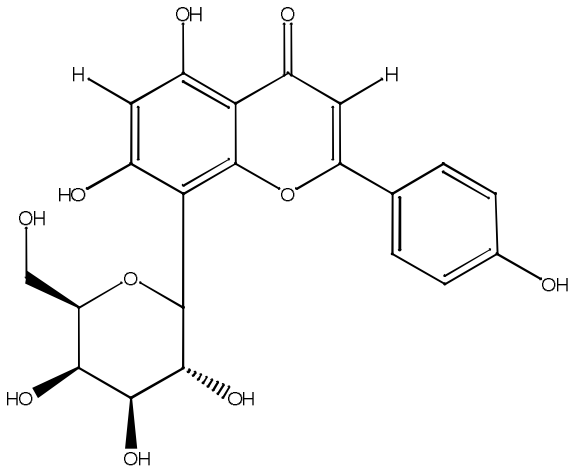

Fig.2. Structure of 8-Glucopyranosyl-4',5,7-trihydroxyflavone

Table 1.^{13}CNMR, ^{1}HNMR and HMBC of 8-Glucopyranosyl-4',5,7-trihydroxy flavone

No	^{13}C-NMR (δ, ppm)	^{1}H-NMR (δ_H, ppm)	HMBC (δ_C, ppm)
2	163,85	-	
3	102,39	6,76 (*s*)	103,77; 182,01; 163,85; 121,59
4	182,01	-	
5	163,85	-	
6	98,31	6,25 (*s*)	103,77; 160,41; 163,85
7	160,42	-	
8	104,66	-	
9	156,04	-	
10	103,77	-	
1'	121,59	-	
2'	128,96	8,01(*d*)	161,25; 163,85
3'	115,86	6,88 (*d*)	121,59; 161,25
4'	161,25	-	
5'	115,86	6,88 (*d*)	121,59; 161,25
6'	128,96	8,01(*d*)	161,25; 163,85
1"	73,43	4,70 (*d, 8,2*)	104,66; 156,04
2"	70,88	3,84 (t, 8,2)	73,43; 78,70
3"	78,70	3,27 (m)	70,55
4"	70,55	3,38 (t)	78,70
5"	81,84	3,26 (m)	70,55
6"	61,30	3,52 (m) 3,77 (*d, 11,5*)	

Table 2. Antioxidant activities of extracts with vit C as standart control

Sample	**Absorbance (λ = 515 nm)**	**Concentration (µg/mL)**	**% Inhibition**	**IC_{50} (µg/mL)**
Blank	0,755			
Vitamin C	0,4978	4	34,07	6,92
	0,4049	6	46,37	
	0,3554	8	52,93	
	0,2545	10	66,29	
	0,1440	12	80,93	
Metanol Extract	0,5676	5	24,82	53,11
	0,5162	10	31,63	
	0,4587	25	42,83	
	0,2842	50	54,98	
	0,2622	100	65,27	
n-hexane Extract	0,6657	5	11,83	256,23
	0,6452	10	14,54	
	0,6352	25	15,87	
	0,5852	50	22,49	
	0,5657	100	26,07	
Ethyl acetate Exract	0,5764	5	23,66	57,96
	0,5292	10	29,91	
	0,4266	25	43,50	
	0,3781	50	49,92	
	0,3263	100	63,78	
n-buthanol Extract	0,6525	5	13,58	132,39
	0,6030	10	20,13	
	0,5743	25	23,93	
	0,5340	50	29,27	
	0,5025	100	40,86	

Table 3. Antioxidant activities of 8-Glucopyranosyl-4',5,7-trihydroxyflavone (**I**)

Sample	**Absorbans (λ = 515 nm)**	**Concentration (µg/mL)**	**% Inhibisi**	**IC_{50} (µg/mL)**
Blank	0,7634			
(1)	0,7162	5	6,18	68,07
	0,6440	10	15,64	
	0,5371	25	29,64	
	0,3878	50	49,20	
	0,2822	100	63,03	

Antioxidant activity

The free radical scavenging activity was tested as leching of the stable 1,1-diphenyl-2picrylhydrazil radical (DPPH). In its radical form, DPPH has an absorption band at 515 nm, which disappears upon reduction by an antiradical compound. The reaction mixture (2mL) contained 500µL of daily prepared DPPH solution (1mM) and various concentrations of tested compound or of standart reference dissolve in MeOH. After 30 min in the dark at room temperature, the absorbance was recorded at 515 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (Table 2 and Table 3).

$$\text{DPPH Scavenging Effect (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} X\ 100$$

Antioxidant activity measured using DPPH accounts partially for the bound and insoluble antioxidants. Relative antioxidant content provides an indication of importance of each of the extracts. The extracts of methanol and ethyl acetate more potent antioxidant activity when compared with extracts of n-hexane and n-butanol. Isolation of ethyl acetate extract obtained compound 8-Glucopyranosyl-4 ',5,7-trihydroxyflavone which have antioxidant activity of 68.07µg/mL. In another study, these compounds are found also in *Basella rubra*Linn[4].

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