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# Antioxidant Flavone C-Glycoside from *Epipremnopsis media* (Z&M). Engl Plant (Araceae)

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**ABSTRACT**

*The bioassay guided fractionation of methanolic extract from Epipremnopsis media leaves led to the isolation of flavone C-glycoside which Diphenylpicrylhidrazil (DPPH) free radical for antioxidant activity Assay. The flavone C-glycoside was identified as apigenin-8-C-β-D-glucopyranoside, more commonly known as vitexin. The hydrolyzed of the compound yielded isovitexin as its isomer. These structures were elucidated by means of UV, IR, NMR spectroscopic analyses and chemical evidence. In DPPH assays for antioxidant activity showed percentage of inhibition to be 60 % at 100 µg/ml concentration, respectively.*

**Keywords:** Antioxidant Assay Guided, Vitexin, *Epipremnopsis media* (Z&M). Engl *Amydrium medium* (Z&M.), Araceae

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## INTRODUCTION

*Epipremnopsis media* is a scandent herbs belonging to Araceae and mainly found in tropical Southeast Asia. It is popularly known as *Amydrium medium* [1,2]. The infusion of its leaves is used to treat influenza, cancer disease, high blood pressure, and stroke. The ethanol extract of this plant reportedly exhibit various health benefits, including decreasing blood cholesterol and triglyceride and in vitro anticancer activity [3]. Some of these biological effects have been linked to the presence of antioxidant compound in the plant. Previous studies have suggested that extract and the fraction of *Epipremnopsis media* leaves have significant antioxidant activity. Therefore, there is no scientific information on antioxidant properties of this plant. The aims of this research due to investigate antioxidant properties of this plant, isolate and elucidate the molecular structure of the compound responsible for the antioxidant activity. The antioxidant properties will be evaluated by DPPH radical scavenging assay guided. The active fraction was separated continuously, the sub fractions were evaluated by DPPH assays to determine active sub fraction and yield an active compound finally.

## MATERIALS AND METHODS

### General

UV spectra were obtained in Me-OH using Pharmaspec 1700 spectrophotometer (Shimadzu) and IR spectra using FTIR Perkin Elmer (KBr) spectrophotometer. NMR spectra were obtained in DMSO-$d_6$ using Jeol LA-300 NMR

(500 MHz for $^{1}H$ and 125.0 MHz for $^{13}C$) (Jeol, Japan) spectrometers. Chemical shifts were in ppm. The HMQC (Heteronuclear Multi Quantum Coherence) method used the BIRD (Bilinear Rotation Decoupling) pulse sequence and the HMBC (Heteronuclear Multiple Bond Coherence) experiment had a 70 ms long-range coupling delay. Spectra were recorded with an ambient temperature. Silica gel C-60, 40 – 63 μm (Merck®) were used for column chromatography and silika gel 60 PF 254 (Merck®) were used for TLC.

**Plant material**

The leaves of *Epipremnopsis media* (Z&M).Engl were collected from Botanical Garden of Andalas University, Padang, West Sumatra, Indonesia in July 2006. A voucher specimen has been deposited in Herbarium of Andalas University, Coll. Number FAR/03/2005.

**Extraction and Isolation**

The fresh leaves from *E. media* (6 kg) were submitted to extraction with methanol using maceration method. The methanol solution was evaporated under vacuum and afforded the methanolic extract (296,7 g). After dissolution in water, this extract was submitted to liquid-liquid partition with organic solvents: *n*-hexane, ethyl acetate, and buthanol subsequently. The ethyl acetate fractions (17 g) was continued to separate using flash chromatography method and performed on silica Wakogel® C-60 (40-63 μm) 200 gram, Φ 5cm, eluting with *n*-hexane, ethyl acetate, and MeOH (in gradient). This separation was afforded 15 sub fractions (DM-66-(1-14). Mixed fractions (13-14) was separated by silica gel column with ethyl acetate and MeOH (in gradient) as eluent to yield seven Fractions (DM-68-(1-7)). Fraction (DM-68-5) is yellowish powder with brown solutions, this sample were purified through crystallizations with ethyl acetate and methanol and afforded compound DM-69-1 (66 mg).

Compound DM-69-1 : yellow powder; mp 246-248$^{o}$C; UV (MeOH):), $\lambda_{max}$ 270, 332.2nm, $\lambda_{max}$ (NaOMe) : 279.6, 330, 393.8 nm, $\lambda_{max}$ (NaOAc): 279, 354 nm, $\lambda_{max}$ (NaOAc/$H_3BO_3$ ): 271.4, 337.4 nm, $\lambda_{max}$ ($AlCl_3$): 274.2, 341.2 nm, $\lambda_{max}$ ($AlCl_3$/HCl): 278.2, 344.4, 385.8 nm; IR (KBr): $\nu_{max}$ = 3247, 1655, 1569, 1507, 1178, and 833 cm$^{-1}$; $^{1}H$ and $^{13}C$ NMR as shown in Table 1.

Compound DM-69-1 was heated with the mixture of Methanol (2 ml) and HCl (4 N) for 2 h at 80$^{o}$C.

**Antioxidant assay (DPPH assay)**

Stock solutions of each test sample including extract, fractions, purified compound and standard (Gallic acid) were prepared in methanol solutions in concentration of 1 mg/ml for reaction with DPPH free radical. DPPH solution in MeOH (50 μM) was used. Stock solution (200 μl) were mixed with DPPH (3.8 ml) and allowed to stand for 30 min for any reaction to occur. The UV absorbance of these solutions was recorded at 517 nm. The experiment was performed in duplicate and the average absorption was noted for each sample and the percentage of activity was calculated [4].

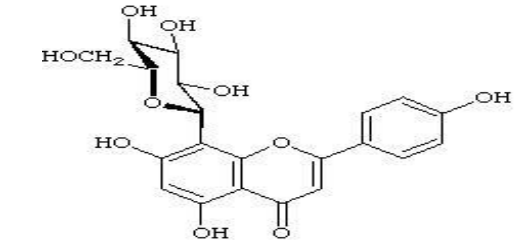
## RESULTS AND DISCUSSION

Compound DM-69-1 was obtained as yellow powder, mp 246-248$^{o}$C, and exhibits a positive magnesium hydrochloric acid test. The absorption bands at $\nu_{max}$ 3247, 1655, 1569, 1507, 1178, and 833 cm$^{-1}$ in the IR spectrum are characteristic of hydroxyl, unconjugated carbonyl, hydrogen bonded carbonyl, and aromatic group respectively. The UV spectrum showed absorption maxima at 270 (Band II), 332.2 (Band I) nm in MeOH and batochromic shift of 61.6 nm in band I ( 4'-OH group), new peak in 330 nm (7-OH) in NaOMe and batochromic shift of 9 nm in band II in NaOAc are indicative of the presence of two free hydroxyl groups at C-7 and C-4'. The batochromic shift of 53.6 nm in band I in $AlCl_3$/HCl are indicative of the presence of hydroxyl group in C-5. There is no hypsochromic shift of $AlCl_3$ spectrum on addition of acid revealed that DM-69-1 was a flavone without ortho-dihydroxyl and 3-hydroxyl groups in the structure. From UV spectral data it is suggested that DM-69-1 has a 5,7,4'-trihydroxylflavone skeleton.

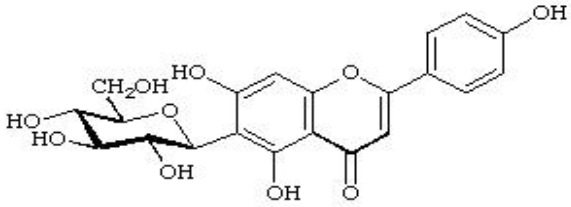
Compound DM-69-1 after hydrolysis obtained two spot in cellulose TLC which the first spot was same with the spot before hydrolysis and the second spot is higher than the first spot. Indicated that hydolysis didn't occurred, the sample was isomerized. From the data revealed that compound is 8-C-Glycocylflavone-5,7,4'-trihydroxyflavone.

As shown in Table 1, the $^{1}H$-NMR spectra of the isolated compound indicated that the presence of a 4'-hydroxyphenyl group [δ 8.03, 6.89 (each 2H, d, *J* = 8.8 Hz)], and two aromatic proton signals at δ 6.27 (s, H-6) and

δ 6.78 (s, H-3). These data indicated that the aglycone moiety was analogous to apigenin. The $^{13}$C-NMR spectrum reveals 21 carbons, which suggested that the structure is a flavonoid containing a saccharide moiety. The six carbon signals of the sugar moiety were at δ 73.88, 71.45, 79.15, 71.06, 82.40, and 61,81, suggesting that DM-69-1 is a flavone C-glycoside. The site of the sugar linkage to the aglycone in DM-69-1 considered to be at C-8 position of 5,7,4'-trihydroxyapigenin, since the signals appeared at δ 105.16 in the $^{13}$C NMR spectrum. It was further confirmed by the appearance of the cross peaks of the anomeric proton of the sugar at δ 4.69 (d, *J* = 10.3 Hz) with the carbons at δ 163.23 (C-7), 105.16 (C-8) and 156.26 (C-9) in the HMBC spectrum. The proton signals at δ 4.69, 3.84, 3.26, 3.37, 3.26, and 3.76 in the sugar moiety were assigned to H-1", H-2", H-3", H-4", H-5" and H-6", respectively by $^{1}$H-$^{1}$H COSY and HMQC. Compared with the corresponding data of the known flavone in the literature, the data identically to vitexin. Thus, the structure of DM-69-1 was determined as apigenin-8-C-β-D-glucopyranoside or vitexin, which has been previously isolated from *Crataegus pinnatifida* and other plant [5]. The hydrolyzed of the compound yielded isovitexin as its isomer (Figure 1). To our knowledge, this the first report on the occurrence of flavone C-glycoside in *Epipremnopsis media.*




Vitexin




Isovitexin

**Figure 1. The structur of vitaxin and its isomer isovitexin [5]**

**Table 1. NMR data of DM-69-1 (DMSO-*d*$_6$)**

| Position | $^{13}$C | $^{1}$H |
|---|---|---|
| 2 | 164.48 | |
| 3 | 102.92 | 6.78 s |
| 4 | 182.65 | |
| 5 | 161.70 | |
| 6 | 98.61 | 6.27 s |
| 7 | 163.23 | |
| 8 | 105.16 | |
| 9 | 156.26 | |
| 10 | 104.55 | |
| 1' | 122.16 | |
| 2', 6' | 129.56 | 8.03 d (8.8) |
| 3', 5' | 116.44 | 6.89 d (8,8) |
| 4' | 160.94 | |
| Glc- 1" | 73.88 | 4.69 d (10.3) |
| 2" | 71.45 | 3.84 t (9.6) |
| 3" | 79.15 | 3.26 m |
| 4" | 71.06 | 3.37 d (8.3) |
| 5" | 82.40 | 3.26 m |
| 6" | 61.81 | 3.76 d (11.8) |

In DPPH assay, the extract and the fraction of *E. media* leaves showed a significant activity. The compound DM-69-1 showed percentage of inhibition to be 27,32 % at 50 µg/ml concentration and to be 60 % at 100 µg/ml, respectively as shown in Table 1. The antioxidant activity of DM-69-1 is a consequence of the presence of the phenolic moieties in the structures. The antioxidant activity of phenolic natural products is predominantly due to their redox properties, *i.e.*, the ability to act reducing agents, hydrogen donors and singlet oxygen quenchers, and to some extent, it could be also be due to their metal chelation potential [6,7].

**Table 2. DPPH Radical Scavenging activity From *E. media* Leaves**

| Sample | % Inhibition (50 µg/ml) |
|---|---|
| MeOH extract | 12.82 |
| *n* – Hexane Fraction | 59.34 |
| EtoAc Fraction | 73.44 |
| BuOH Fraction | 33.88 |
| Gallic acid | 72.66 |

## CONCLUSION

The free radical scavenging property of extract and fraction of *E. media* leaves may explain, at least to some extent, some of the traditional medicinal uses of this plant. While, the pursue for antioxidant compound from the methanolic extract of *E. media* leaves led to the isolation and identification of a flavone C-glycoside.

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