

# Flavon Compound from The Ethyl Acetate Extract of The Stem of Supit (*Tetracera indica* Merr.)

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## Flavon Compound from The Ethyl Acetate Extract of The Stem of Supit (*Tetracera indica* Merr.)

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### ABSTRACT

*Tetracera indica* Merr. in Musi Banyuasin, is one of the traditional medicine used by the community for the treatment of kidney stone disease and gout, but this claim is not recorded in the treatment of kidney stones and gout in Indonesia. In this study, isolation of antioxidant compound from ethyl acetate extracts of supit (*Tetracera indica*) was done. The isolation was carried out through step gradient polarity extraction, and separated and purified by chromatography technique. The determination of the structure of the isolated compound was performed by spectroscopy method including UV, IR, and NMR 1D and 2D, and antioxidant activity was determined by DPPH method. An active antioxidant compound was isolated from ethyl acetate extract in form of yellow solid (15 mg). Based on spectroscopic analysis the isolated compound was 5,8-dihydroxy-7-methoxyflavone. The compound showed strong antioxidant activity ( $IC_{50}$  8.25  $\mu\text{g/mL}$ ) higher than standard ascorbic acid ( $IC_{50}$  11.3  $\mu\text{g/mL}$ ). This data concluded that efficacy of supit (*Tetracera indica*) for the treatment related to antioxidant activity (uric acid) is proven by the identification of one antioxidant compound of this plant.

Key word: Flavon, ethyl acetate extract, *Tetracera indica*, antioxidant

### INTRODUCTION

Indonesia is a country with the second largest diversity of plants in the world, but there are still many medicinal plants that have not been fully utilized (Hanafi, Nina, Zorni, and Nurbaiti, 2005). The use of medicinal plants has been practiced by the Indonesian people for generations. Indonesian herbal medicines are used based on empirical practice: diseases preventive (48.9%) (Hanafi *et al.*, 2005). Although modern medicine has grown tremendously in Indonesia, but traditional medicine is still very popular in rural as well as in urban areas (Elfahmi, Herman, Woerdenbag, and Oliver, 2014). Moreover to having a high diversity of plants, Indonesia is also rich with tribal and cultural diversity (Ernie, 2013). In some tribes, endemic plants was found to be used as traditional treatment. Some times in some tribes are found endemic plants used for traditional treatment.

In previous research has been done exploration and inventory of medicinal plants and their utilization in society based on local resources. One of them is the ethnic Musi in Musi Banyuasin district of South Sumatra. In research of medicinal plants and herbal medicine has been identified 95 species of medicinal plants (Yustian, Muharni, Sukarni,

Zulaicha, and Arbi, 2012). From the 20 selected plants 9 species of them are traditionally used for the treatment of diseases atherosclerosis, diabetes, prostate, gout, and kidney stones. These types of diseases are related to free radical activity (Paravicini and Touyz, 2008). *In vitro* antioxidant activity test of 9 selected plant extracts is one of them is supit stems, showed high antioxidant activity with value of % inhibition 69.6% at test concentration 1000 ppm. Based on literature study of extracts that showed antioxidant activity > 50% categorized as active source of antioxidant compound (Chaudhary, Bhandari, and Pandurangan, 2011; Muharni, Fitrya, and Nurmaliana, 2019).

Plants produce a wide diversity of secondary metabolites exhibit and a wide array of biological and pharmacological properties (Michalek 2015). Secondary metabolites from plants are the basis for many drugs currently used to treat various diseases, including diseases related to radical activity (Rogerio, Sá-Nunes, and Faccioli, 2010). Phytochemical tests of ethanol extract of supit (*Tetracera indica*) have been performed and exhibited positive phenolic compounds. Phenolic compounds are generally active as antioxidant (Kenari, Mohsenzades, and Amiri, 2014; Brewer, 2011; Kumar, Mishra, and Pandey,

2013). The limited ingredients available medicines has prompted researchers to explore the potential of nature to find new bioactive compounds.

Chemical compounds from leaf of supit (*Tetracera indica*) has been reported 4 terpenoid compounds:  $\beta$ -sitosterol, lupeol, Betuline, betulinic acid and 6 compounds flavonoid 5, 7-dihydroxy-8-methoxylavone or another name (Wogonin), 5, 7,8-trihydroxyflavone, isocutellarein methyl ether, kaemferol, quersetin, tetrokrisin (Dogarai, 2011). The methanol extract leaf of supit showed activity as antidiabetic (Ahmed, Dogarai, Amiroudine, Taher, Latip, Umar, and Muhammad, 2012). Other research Fitriya, Muharni, and Maretha (2012) reported one phenolic compound from ethyl acetate extract of fruit.

In another study of *Tetracera indica* leaf extract containing flavonoid wagonin compound was active as an antibacterial (Lima, Lemos, and Conserva, 2014). Abdullah, Ismail, Jamaludin, and Mashim (2013) reported that the stems of supit show activity as antihyperuricemia, antidiabetic and anti-inflammatory and two compound has been identified of stem supit that is betulinic acid and 5,7-dihydroxy-8-methoxyflavone. In this paper will be reported antioxidant compound active by DPPH method from ethyl acetate extract stem of supit.

## EXPERIMENTAL SECTION

### Material

The stem of *Tetracera indica* (2 kg) were collected from Ngulak I village Nisi Banyuasin South Sumatera in June 2017. The botanical identification of the samples was confirmed by Dr. Laila Hanum. A voucher specimen (number VIC 2701) was deposited at the Herbarium Department Biology Sriwijaya University. Material for isolation: silica gel 60 (70 -230 mesh.), thin layer chromatography (TLC) using Merck (Art.5554), silica gel G 60 (70 - 230), methanol p.a, n-hexane p.a, ethyl acetate p.a, chloroform p.a acetone p.a, distilled water. Reagent for antioxidant activity: DMSO (dimethyl sulfoxide), DPPH (1,1-diphenyl-2-picrylhydrazyl), and ascorbic acid.

### Instrumentation

The apparatus in the research were rotary evaporator R-114 Buchi, column

chromatography(CC), ultra-violet lamps CAMAG 254 nm, radiation ( $\lambda = 254$  and 365 nm). Fisher Jhon melting point apparatus, spectrophotometer ultraviolet Beck DU-7500. FTIR-Perkin Elmer, NMR JEOL JNM ECA-500 spectrometer. UV-visible spectrophotometer using a single beam provided by Shimadzu-UV mini 1240 instrument, and commonly used glassware in the organic laboratory.

### Preparation of Extracts

The stem of *Tetracera indica* were dried at 30 °C for 20 days with continuous moisture monitoring. After the material was completely dry, it was pulverized in a knife grinder, obtained 550 g of sample. The dried stem bark were subjected to exhaustive extraction in maceration apparatus using an increasing polarity solvent system, with n-hexane, ethyl acetate and ethanol as solvents each for 24 h. The extracts were then concentrated at reduced pressure used Rotary evaporator. Each of the extracts was tested antioxidant activity.

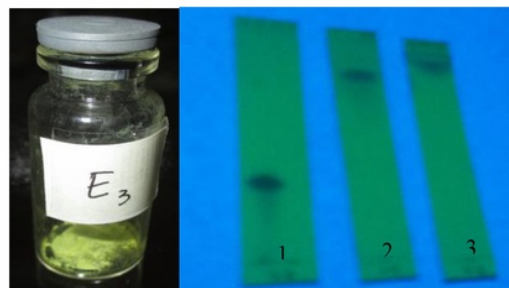
### Evaluation of Antioxidant Activity of Each Extract

The antioxidant activity test was performed using DPPH radical-scavenging activity (Selvi, Joseph, and Jayaprakasha, 2003; Tuanjai, Supalax, Thawatchai, and Wittaya, 2011). The concentration series for extract :1000, 500, 250, 125, 62.5 and 31.25  $\mu\text{g/mL}$  (in DMSO) was prepared. 0.2 mL aliquot of sample solution was mixed with 3.8 mL of DPPH (0.2 mM in methanol) where 1.98 mg DPPH was placed on a 100 mL volumetric flask then add methanol until the volume 100 mL. This mixture was shaken at room temperature for 30 min.

The absorbance of the mixture was then measured by UV-Vis spectrophotometer at  $\lambda_{\text{max}}$  517 nm (Selvi *et al.*, 2003). For positive control (standard) used ascorbic acid and negative control (blank) used methanol. Isolated compound solution was analyzed in triplicate. The radical-scavenging activity was evaluated as the percentage of inhibition according to the following equation: %inhibition= [(absorbance of control - absorbance of sample)/ absorbance of control]x 100 (Delwar, Shahid, Masur, Shohel, Shahid, and Mohammad, 2014) and the average values were plotted to obtain the  $\text{IC}_{50}$  against DPPH by linear regression.

**Table 1.** Antioxidant activity of each extract

Concentration	Extract n-hexane		Extract Ethyl acetate		Extract Ethanol	
	Absorbance	% inhibition	Absorbance	% inhibition	Absorbance	% inhibition
1000	0.577	29.63	0.081	90.12	0.047	94.27
500	0.589	28.17	0.140	82.93	0.052	93.65
250	0.590	28.05	0.353	56.95	0.063	92.32
125	0.592	27.80	0.469	42.80	0.074	90.97
62.5	0.620	24.39	0.534	34.88	0.075	90.85
31.5	0.679	17.20	0.547	33.29	0.078	90.49



**Figure 1.** TLC isolated compound by eluents : n-hexane : ethyl acetate 9 : 1 (1) ; 5 : 5(2) and EtOAc : MeOH 9.5 : 1.5 (3).

#### Isolation and Purification <sup>6</sup>Antioxidant Compound from Ethyl Acetate Extract

Ethyl acetate extract (3.5 g) was separated by silica column chromatography using silica G 60 F<sub>254</sub> (230-400 mesh), eluted with gradient polarity mixtures of hexane-ethyl acetate (9:1 - 5:5) were collected. Base on TLC analysis merged into six fraction F1 (vials 1 - 5; 25 mg), F2 (vials 7 - 23; 1300 mg), F3 (vials 26 - 67; 250 mg), F4 (vials 68 - 80; 500 mg), F5 (vials 81 - 84; 100 mg), and F6 (vials 85 - 95; 700 mg). From the selected fractions, the 4<sup>th</sup> fraction (500 mg) was further purified by re-chromatography using silica gel 60 (70 - 230 mesh), eluted with gradient polarity mixtures of n-hexane-ethyl acetate (9:1 - 7:3) each 150 mL.

Base on TLC analysis to obtained four sub fractions F<sub>4.1</sub> (vials 1 - 3; 23 mg), F<sub>4.2</sub> (vials 4 - 8; 44 mg), F<sub>4.3</sub> (vials 9 - 14; 84 mg) dan F<sub>4.4</sub> (vials 15 - 44; 194 mg). Sub fraction F<sub>4.3</sub> was re-chromatography using the same method (silica <sup>39</sup>, eluted with gradient polarity mixtures n-hexane - EtOAc (9:1) to yield three fraction F<sub>4.3.1</sub> (15 mg), F<sub>4.3.2</sub> (50 mg). Fractions F<sub>4.3.1</sub> obtained yellow solid (15 mg) and after analyzed with TLC showed one spot so it was pure compound.

#### Elucidation Structure

The structure of the isolated compound was elucidated using UV, IR, NMR 1D (<sup>1</sup>H and <sup>13</sup>C NMR, DEPT), and NMR 2D (HMQC,

<sup>1</sup> and HMBC) spectroscopy and by comparison with data from the literature.

#### Evaluation Antioxidant Activity of Isolated Compound

The pure isolated compound was determined <sup>38</sup> antioxidant activity by the same procedure as the antioxidant activity assay of the extract but using the concentration 100, 50, 25, 12.5 and 6.75 µg/mL.

#### RESULTS AND DISCUSSION

The stem of supit (2 kg) after dried obtained 550 g of powder. The powder *Tetracera indica* of 550 g was extracted with n-hexane solvent, ethyl acetate and ethanol respectively and after concentrated was obtained n-hexane extract 250 mg, ethyl acetate 3,50 g and ethanol extract 3.7 g. Each extract, has been done antioxidant activity test with varying concentration (**Table 1**). Mariod, Matthauss <sup>16</sup> Hussein, (2008) state that the value of antioxidant activity is determined based on the value of percentage inhibition, the higher the value of the inhibition, the higher the antioxidant activity.

**Table 1** showed that at <sup>45</sup> the same concentration 1000 µg / mL, the ethanol extract showed the highest activity with inhibition 94.27% followed by ethyl acetate extract with inhibition 90.12%. Meanwhile, n-hexane extract only gives an inhibition of

29.63%. Extracts are categorized potent containing antioxidant compounds which have percent inhibition > 50% at a concentration of 1000  $\mu\text{g/mL}$  (Chaudhary *et al.*, 2012). Based on this data it is stated that the extract of ethyl acetate and ethanol extract from stems of supit (*Tetracera indica*) potentially contain antioxidant compounds. The Compounds that exhibit antioxidant activity generally provide fluorescent under UV light at a wavelength of  $\lambda$  365 nm. Ethyl

acetate extract after separation and purification a compound was isolated from the ethyl acetate extract of stem *T. indica*. The isolated compound was as a yellow solid (15 mg). The purity test of the isolated compound was carried out by TLC analysis using various eluents : n-hexane : ethyl acetate (9 : 1 ; 5 : 5) and EtOAc : MeOH (9.5 : 1.5) showed a single spot on the UV lamp at  $\lambda$  365 nm (Figure 1).

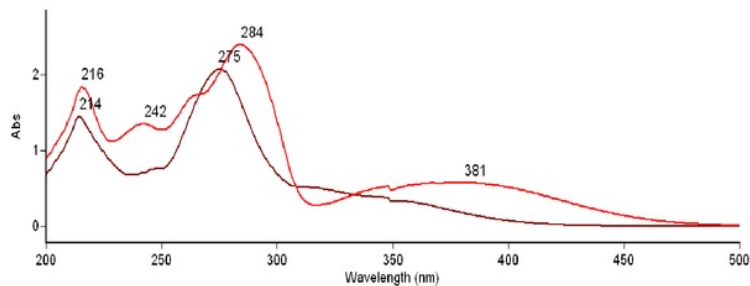


Figure 2. UV Spectrum of isolated compound.

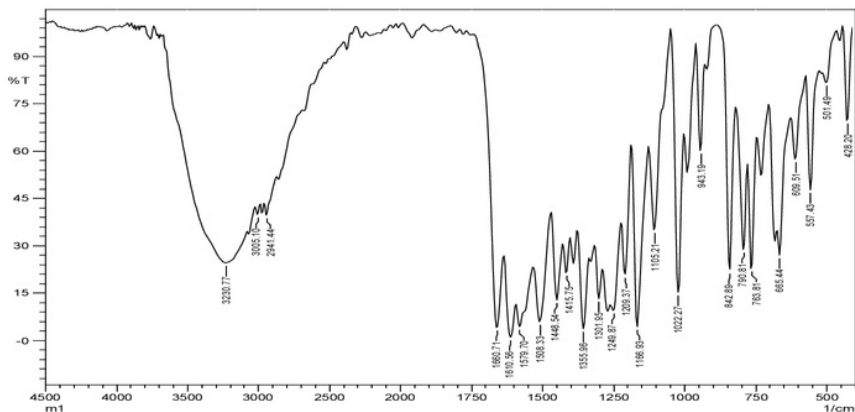


Figure 3. IR spectrum of isolated compound.

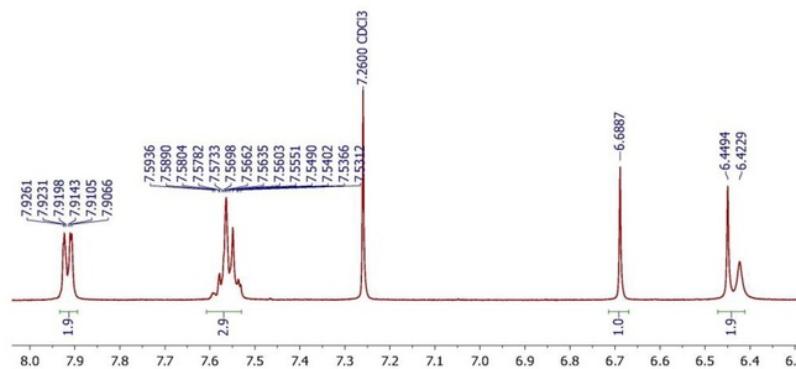


Figure 4. Spectra  $^1\text{H}$  NMR of isolated compound

The purity test of isolated compound were also conducted with measurement of the melting point. The measurement result obtained isolation compound have melting point is 203-205 °C, the narrow melting point range ( $\leq 2$  °C) showed the isolated compound was pure.

#### Determination Structure of The Isolated Compound

Structural determination was done through spectroscopic analysis using several methods of spectroscopy UV, IR, NMR 1D ( $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, dan DEPT) and NMR 2D (HMBC, HMQC). The UV spectrum (Figure 2) shows the maximum absorption at  $\lambda_{\text{max}}$  275 nm which is the absorption for the conjugated C = C bond. The addition of MeOH as a shear reagent causes a maximum wavelength shift to  $\lambda_{\text{max}}$  284 nm, indicating the presence of OH phenolic. The measurement of the infrared spectra of the isolated compound with KBr pellet shows the absorption on  $\nu_{\text{max}}$   $\text{cm}^{-1}$  3230.77 with a wide peak shape indicating that the OH group having hydrogen bond, 1660.71 (conjugated C=O), 1415.76; 1508.33; dan 1448.54 (C=C conjugation), and 1166.93 (C-O ether). Based on the absorption of IR, it is suspected that the isolated compound is a flavonoid group in which literature study the

IR for flavonoids such as morelloflavon as reported by Muharni, Elfitra, and Amanda (2011) provides absorption on  $\nu_{\text{maks}}$  3263 (OH group), 1643 (conjugated C=O), 1552; 1514; 1439 (C=C aromatic) and 1180  $\text{cm}^{-1}$  (C-O ether).

The  $^1\text{H}$  NMR spectrum of the isolated compound (Figure 4) show the proton signal of methoxy at  $\delta_{\text{H}}$  4.04 (3H, s) and the signal of the aromatic proton at the  $\delta_{\text{H}}$  6.0-8.0 ppm. Signals at  $\delta_{\text{H}}$  6.43 (1H, *brs*) with widened peaks are signals for phenolic OH. Signals at  $\delta_{\text{H}}$  6.45 ppm (1H, s) and 6.69 (1H, s) are 2 non-split aromatic protons, 7.54 - 7.56 (3H, *m*) are 3 proton aromatics that split with protons at  $\delta_{\text{H}}$  7.91 (2H, *dd*  $J = 3.85$ ) and signal at  $\delta_{\text{H}}$  9.49 (1H, s) are signal for proton OH chelate. The  $^{13}\text{C}$  NMR spectrum of the isolated compound showed 14 signals. Signal  $\delta_{\text{C}}$  62.2 ppm is characteristic signal for methoxy carbon. Signals at  $\delta_{\text{C}}$  99.00 - 165 ppm are signals for C SP<sup>2</sup> from aromatic carbon. The signal at  $\delta_{\text{C}}$  182.4 ppm is a signal for C carbonyl in the form of a ketone. In HMQC spectrum (Figure 6) showed proton methoxy at  $\delta_{\text{H}}$  4.04 ppm bound to carbon  $\delta_{\text{C}}$  62.2 ppm and on spectrum of HMBC showed correlation with carbon at  $\delta_{\text{C}}$  127.0 ppm.

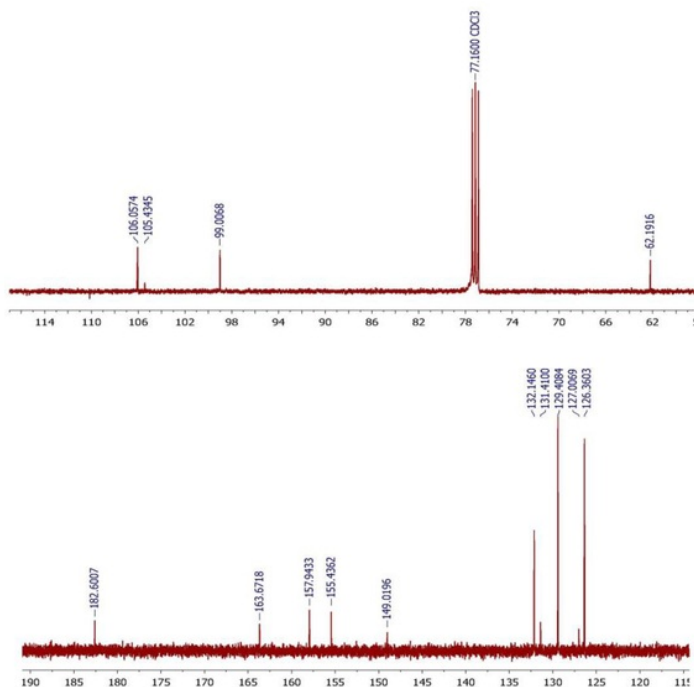
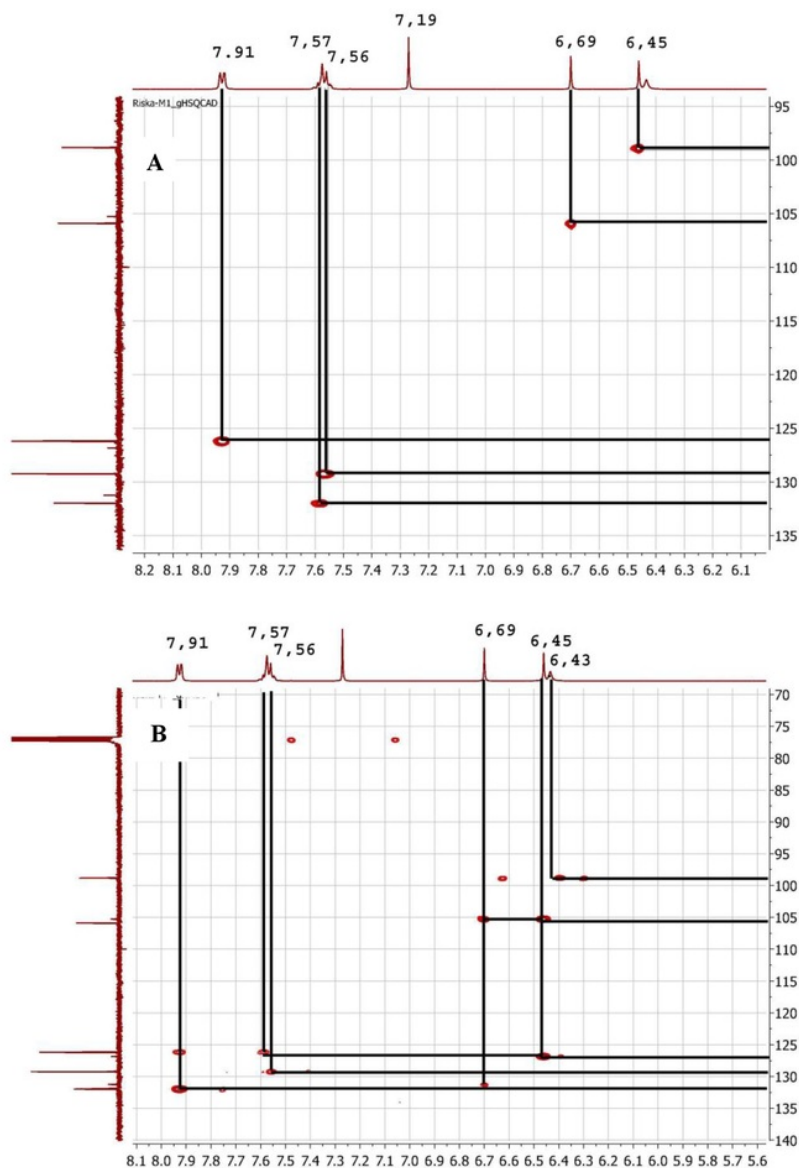


Figure 5. Spectrum  $^{13}\text{C}$  NMR isolated compound.

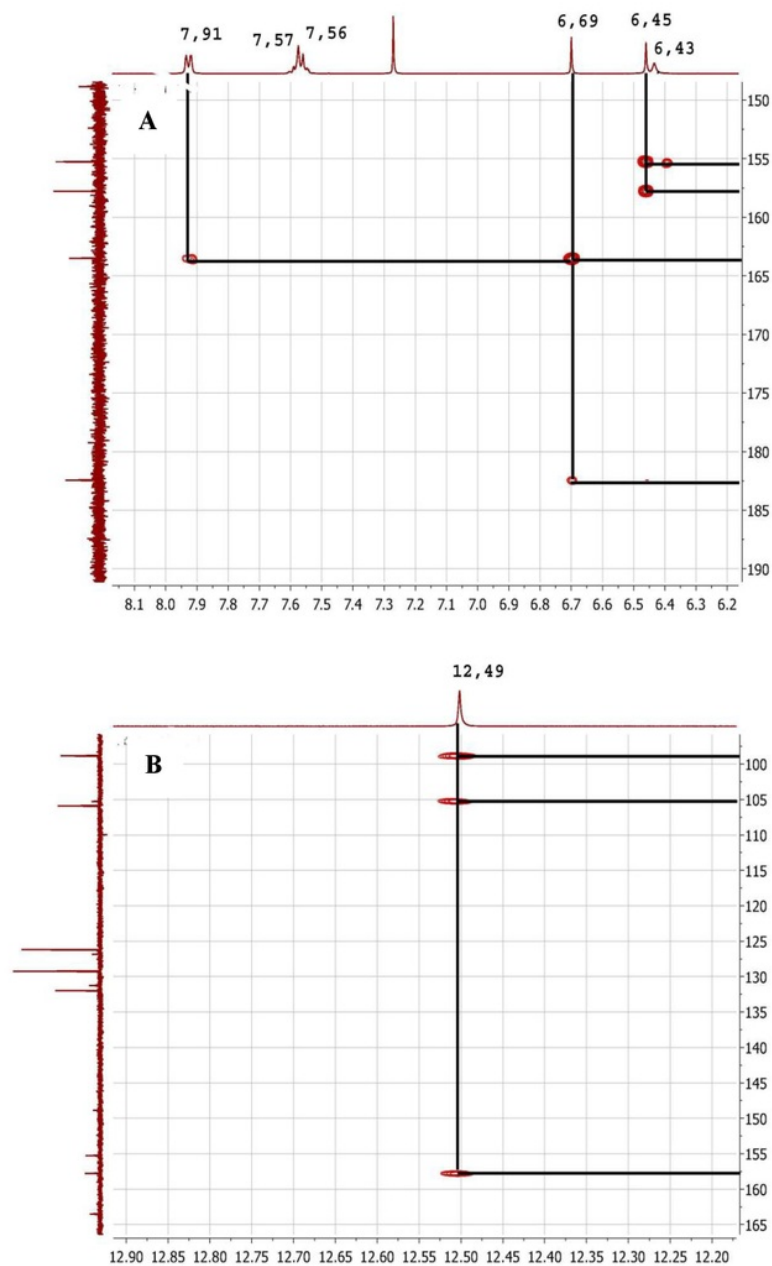


**Figure 6.** Spectrum HMQC at  $\delta_H$  3.2 – 8.4  $\delta_C$  60 -135 ppm (A) and HMBC at  $\delta_H$  5.6 -8.1  $\delta_C$  700 -140 ppm (B)

The HMQC spectrum also showed proton at  $\delta_H$  6.45 ppm (1H, s) and  $\delta_H$  6.69 (1H, s) respectively bound to carbon at  $\delta_C$  99.0 ppm and  $\delta_C$  105.4 ppm. In the HMBC spectrum (Figure 6) showed protons at  $\delta_H$  6.45 ppm (1H, s) correlated with carbon at  $\delta_C$  106.1(C10), 127.0 (H8) 155.4 (C7), 157.9 ppm (C9). Meanwhile the proton at  $\delta_H$  6.69 (1H, s) correlated with carbon at  $\delta_C$  106.1 (C10), 131.4 (1') 163.5 (C2), 182.4 ppm (C4). This indicates the carbon at  $\delta_C$  106.1 ppm was

located between the carbon at  $\delta_C$  99.0 and 105.4 ppm.

The HMQC spectrum also showed proton at  $\delta_H$  7.56 (2H) ppm, 7.57 (1H, s) and 7.91 ppm (2H) respectively bound to carbon respectively  $\delta_C$  126.4 132.2 and 129.4 ppm. Based on the integration of the aromatic proton  $\delta_H$  7.56 and 7.91 respectively 2 protons then it is assumed the carbon signals at  $\delta_C$  126.4 and 129.4 ppm were signals each representing 2 carbon.



**Figure 7.** Spectrum HMBC at  $\delta_H$  6.2 – 8.1 ppm and  $\delta_C$  12.2 – 12.9 ppm and  $\delta_H$  100 –165 ppm.

Based on this HMQC data the number of carbon signals is 16 signals and the compound has an aromatic group in the form of mono substitution. In the HMBC spectrum the proton signals at  $\delta_H$  7.56 ppm correlate with the carbon at 131.4(1'), 129.4 ppm (C3',5'), meanwhile the signal at  $\delta_H$  7.91ppm Showed

correlated with carbon at  $\delta_C$  126.4( 2',6'), 132.1 ppm (C4'). This suggests that the protons at  $\delta_H$  7.56 and  $\delta_H$  7.91 ppm were in adjacent C positions.

Furthermore, it was also seen in the HMQC spectrum where proton at  $\delta_H$  12.49 ppm bound to carbon at  $\delta_C$  163.76 ppm and on



the HMBC spectrum this proton visible correlated with carbon at  $\delta_c$  99.0; 105.4 and 157.9 ppm  $\delta_c$  99.0; 106.1 ppm. This indicates that this hydroxyl proton is a chelated proton adjacent to a proton  $\delta_H$  6.45 ppm. The correlation between proton and carbon is shown in **Figure 8**. Based on UV IR, NMR 1d and 2 D spectroscopy data, it was concluded that isolation compound was flavonoid 5,8-dihydroxy-7-methoxyflavone with molecular formula  $C_{16}H_{12}O_5$  with the structure shown in **Figure 8**.

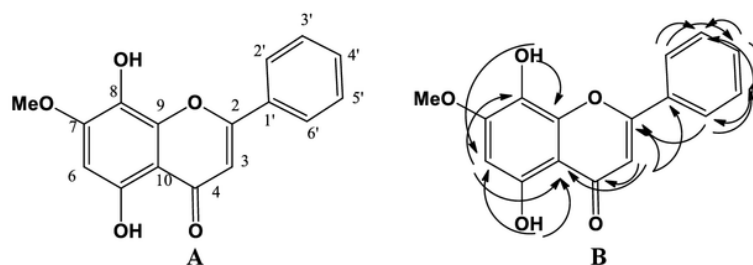
#### Antioxidant activity of isolated compound

Antioxidant activity test was done by DPPH method with concentration variation 100, 50, 25, 12.5 and 6.75 ppm. Antioxidant activity is determined based on the value of % inhibition (**Table 3**). The higher the % inhibition, the stronger the antioxidant activity

**Table 3** showed the higher the concentration of the test the smaller the absorbance and the percent inhibition value will also be higher. Based on the percentage of inhibition seen at a concentration of 6.25  $\mu\text{g} / \text{mL}$ , the isolation compound results showed higher activity than the standard ascorbic acid. To determine the value of  $IC_{50}$ , the plot curve between concentration and the value of % inhibition based on the linear regression. Based on regression linear obtained equation shows that  $IC_{50}$  value of the isolated compound is 8.25  $\mu\text{g} / \text{mL}$ , while the ascorbit acid gives  $IC_{50}$  value of 11.3  $\mu\text{g} / \text{mL}$ . Based on this data concluded the efficacy of supit plants (*Tetracera indica*) for the treatment related to antioxidant activity (uric acid) is proven by the identification of one antioxidant compound of this plant. Isolatet compound is flavonoid group.

**Table 2.** Chemical shift data of proton and carbon of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of isolation compound at 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$  in  $\text{CDCl}_3$ .

No C	$\delta_c$ (ppm)	$\delta_H$ (ppm), integration, multiplicity, J (Hz)	HMBC
2	163.7		
3	105.4	6.69 (1H, s)	106.1 (C10), 131.4 (1'), 163.5(C2), 182.4 (C4)
4	182.4		
5	149.0	12.49 (1H,s)	
5-OH	12.69		99.0 (6), 106.1 (10)
6	99.0	6.45(1H, s)	106.1(C10), 127.0 (H8), 155.4 (C7), 157.9 (C9)
7	155.4		
7-OMe		4.24(3H, s)	127.0 (C8)
8	127.0		
8-OH		6.43 (1H, brs)	99.0(C6), 127.0(C8)
9	157.9		
10	106.1		
1'	131.4		
2',6'	126.4	7.56 (2H, m)	131.4(1'), 129.4(C3',5')
3',5'	129.4	7.91 (2H, m)	126.4(2', 6'), 132.1 (C4')
4'	132.1	7.57 (1H, m)	126.4 (C2')



**Figure 8.** HMBC correlation (A) and structure isolation compound (B)

**Table 3.** Antioxidant activity of isolated compound

No	Concentration	Isolated compound		Ascorbic acid	
		Absorbance	% inhibition	Absorbance	% inhibition
1	100	0.017	94.74	0.032	94.2
2	50	0.019	94.12	0.082	85.0
3	25	0.084	73.99	0.169	69.2
4	12.5	0.091	71.83	0.275	49.8
5	6.25	0.218	32.5	0.375	31.57

Many flavonoids are shown to have antioxidant activity, such as for the prevention of coronary heart disease, hepatoprotective activity, anti-inflammatory, and anticancer (Kumar and Pandey, 2013).

The isolated compound 5,8-dihydroxy-7-methoxyflavone was known by another name wogonin and has been previously found on the leaf extract (Dogarai *et al.*, 2011) and stem extract of *Tetracera indica* (Abdullah *et al.*, 2013), but is first reported as active antioxidant against DPPH radicals.

**CONCLUSIONS**

The flavon compound has been isolated from ethyl acetate extract of the stem of supit (*Tetracera indica*) and identified as 5,8-dihydroxy-7-methoxyflavone. This compound showed strong antioxidant activity by DPPH method with IC<sub>50</sub> value 8.25 µg / mL.

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