

## SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF 7-HYDROXY-3',4'-DIMETHOXYFLAVONE

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### ABSTRACT

Synthesis of flavones and their derivatives has attracted considerable attention due to their significant pharmaceutical effects. 7-hydroxy-3',4'-dimethoxyflavone has been synthesized and its antioxidant activity has been investigated. Flavone was synthesized by oxidative cyclization of chalcone. 2',4'-dihydroxy-3,4-dimethoxychalcone was prepared by Claisen-Schmidt condensation of 2,4-dihydroxyacetophenones with 3,4-dimethoxybenzaldehydes in the presence of aqueous solution of sodium hydroxide and ethanol at room temperature. Oxidative cyclization of 2',4'-dihydroxy-3,4-dimethoxychalcone was done by using I<sub>2</sub> catalyst in DMSO to form 7-hydroxy-3',4'-dimethoxyflavone. The synthesized compounds were characterized by means of their UV-Vis, IR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectral data. The compound was tested for their antioxidant activities by DPPH method.

**Keywords:** Flavone; chalcone; Claisen-Schmidt condensation; Antioxidant activity

### ABSTRAK

Sintesis flavon dan turunannya telah menjadi penelitian yang menarik karena efek farmakologinya. 7-hidroksi-3',4'-dimetoksiflavon telah disintesis dan diuji aktivitas antioksidannya. Flavon ini disintesis melalui siklisasi oksidatif senyawa kalkon. 2',4'-dihidroksi-3,4-dimetoksikalkon disintesis melalui kondensasi Claisen-Schmidt dari 2,4-dihidroksiasetofenon dengan 3,4-dimetoksibenzaldehid dalam larutan NaOH dan etanol pada suhu ruang. Siklisasi oksidatif 2',4'-dihidroksi-3,4-dimetokskalkon dilakukan menggunakan katalis I<sub>2</sub> dalam DMSO untuk membentuk 7-hidroksi-3',4'-dimetoksiflavon. Senyawa hasil sintesis dikarakterisasi berdasarkan data spektrum UV-Vis, IR, <sup>1</sup>H-NMR dan <sup>13</sup>C-NMR. Senyawa tersebut diuji aktivitas antioksidan menggunakan metode DPPH.

**Kata Kunci:** Flavon; kalkon; kondensasi Claisen-Schmidt; aktivitas antioksidan

### INTRODUCTION

Flavones occur in nature in free state with varieties of substitution pattern. Synthesis of flavones becomes centre of attraction for many scientist because of their various physiological and pharmacological properties, such as antibacterial and antifungal [1-2], anti-inflammatory [3], antimicrobial [4]. Also antioxidant, antiviral, antiproliferative, cardiovascular effects of flavones have been reported in literature [5]. Having such a varied pharmacological activity and synthetic utility, flavones have attracted chemists to develop newer strategies for their synthesis.

Several strategies for the synthesis of flavones have been reported. The main synthetic methods include the Allan-Robinson Synthesis, the Baker-Venkataraman

rearrangement, synthesis from chalcones, and several other more recent methods. In Allan-Robinson reaction, o-hydroxyaryl ketone was reacted with anhydride aromatic in order to form the flavone or isoflavone [6]. In Baker-Venkataraman reaction, 2-hydroxyacetophenone was converted to ester, which then underwent rearrangement by intramolecular Claisen condensation in the presence of potassium hydroxide and pyridine to afford 1,3-diketone. This compound was then cyclised to flavone under rather harsh conditions for either by treatment with concentrated sulfuric acid or heating with glacial acetic acid [7]. Among them, oxidative cyclization of chalcones still occupies prominent position. Oxidative cyclization of 2'-hydroxychalcones constitutes an important route for the synthesis of flavones and number of oxidizing agents such as oxalic

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acid [8], DDQ [2], microwave irradiation [9], sodium tellurite [10], and SOCl<sub>2</sub>/EtOH [11] have been reported in literature for this conversion, but these often require longer reaction time, high temperature requirements, highly acidic conditions, low selectivity, toxic reagents, and formation of mixture of product containing flavones, flavanones and aurones have been reported in some cases. Although, several modifications had been made to counter these problems, there is still a need for the development of selective and better strategies for the synthesis of flavone. By using I<sub>2</sub>-DMSO as oxidizing agent in oxidative cyclization of chalcones as convenient alternative to the formation of flavone will be developed. This method were done to oxidation of 2'-hydroxychalcone [12], synthesis of 2',4',6'-trioxygenated flavones [4], and 2'-oxygenated flavones [13].

The hydroxychalcone was prepared by Claisen-Schmidt condensation of 3,4-dimethoxybenzaldehydes with 2,4-dihydroxyacetophenones in the presence of aqueous solution of sodium hydroxide and ethanol at room temperature. 3,4-dimethoxybenzaldehydes was synthesized by eugenol via formation of isoeugenol and vanillin. Eugenol is main component of clove oil, abundantly present in Indonesia so this study is organic synthesis approach based on natural product. The synthesis of chalcone proceeded smoothly without protection of the hydroxy group of acetophenone to give 2',4'-dihydroxy-3,4-dimethoxychalcone. The second step, cyclization of chalcone were done efficiently by using I<sub>2</sub>-DMSO as oxidizing agent to form 7-hydroxy-3',4'-dimethoxyflavone.

## EXPERIMENTAL SECTION

### Materials

All chemicals and solvents (2,4-dihydroxyacetophenone, 3,4-dimethoxybenzaldehyde, sodium hydroxide, ethanol, HCl, ether, anhydrous Na<sub>2</sub>SO<sub>4</sub>, silica gel, Iodine, dimethyl sulfoxide, EtOAc, CDCl<sub>3</sub>, TMS, sodium thiosulphate, KBr pellets, n-hexane, acetone) were purchased from E-Merck.

### Instrumentation

Column chromatography was performed on Merck silica gel 60 (0.063-0.200 mm) to ensure homogeneity of the title compounds. Completion of reaction and purity of the final compounds were checked by TLC (E. Merck). Further, the authenticities of the compounds were established by UV-Vis, IR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra. UV-Vis spectra was measured on spectrophotometer UV-Vis (UV, Milton Roy - Spectronic 3000). IR spectra was recorded on a Fourier-transform

infrared spectrometer (Shimadzu FTIR-8201 PC) in KBr pellets. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were performed JEOL JNM ECA500 instrument using CDCl<sub>3</sub> as solvent and Tetramethyl silane (TMS) as internal reference.

### Procedure

#### Synthesis of 2',4'-dihydroxy-3,4-dimethoxychalcone

A mixture of 2,4-dihydroxyacetophenone (0.01 mole) and 3,4-dimethoxybenzaldehyde (0.01 mole) were stirred in ethanol (15 mL) and then an aqueous of sodium hydroxide (12 mL) were added. The reaction mixture was stirred at room temperature and left at room temperature for 48 h. After that, the reaction mixture was poured into iced water, acidified with cold HCl (10%), extracted with ether (3 x 25 mL). The ether layer was washed with water, followed by drying over anhydrous Na<sub>2</sub>SO<sub>4</sub> and the solvent was evaporated. The reaction mixture was purified by Column chromatography on a silica gel column (n-hexane : acetone, 7:3). The product was recrystallized from ethanol as orange needles (1.2 g; 40%), and characterized by UV-Vis, IR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR Spectroscopy.

#### Synthesis of 7-hydroxy-3',4'-dimethoxyflavone

A solution of 2',4'-dihydroxy-3,4-dimethoxychalcone (3 g, 0.01 mmole) and iodine (250 mg) in 10 mL DMSO was refluxed for 1 h. The mixture was poured into water, and the resulting syrup was extracted with EtOAc (3 x 15 mL). The organic phase was washed with sodium thiosulphate solution (20%), water and brine. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and the solvent was then evaporated under reduced pressure. The residual syrup was chromatographed on a silica gel column with (n-hexane : acetone, 7:3) as eluent and the compound purified by recrystallized from ethanol as yellow needles (0.2 g; 33%). The product was characterized by UV-Vis, IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR Spectroscopy.

#### Antioxidant activity of Compounds was performed by DPPH method

The various concentrations of Compounds (200, 220, 240, 400, 500 µg/mL) were prepared. Stock solution of DPPH 0.4 mM in ethanol was prepared. 50 µL samples add with 1.0 mL solution of DPPH and 3.95 mL of ethanol. After vortexing, the mixture was incubated for 30 min at room temperature and the absorbance at 517 nm was measured. The difference in absorbance between a test sample (50 µL extract and 4.95 mL ethanol) and control (1.0 mL and 4.0 mL ethanol) was considered as activity. The activity was shown as IC<sub>50</sub> value. All values are shown as mean of

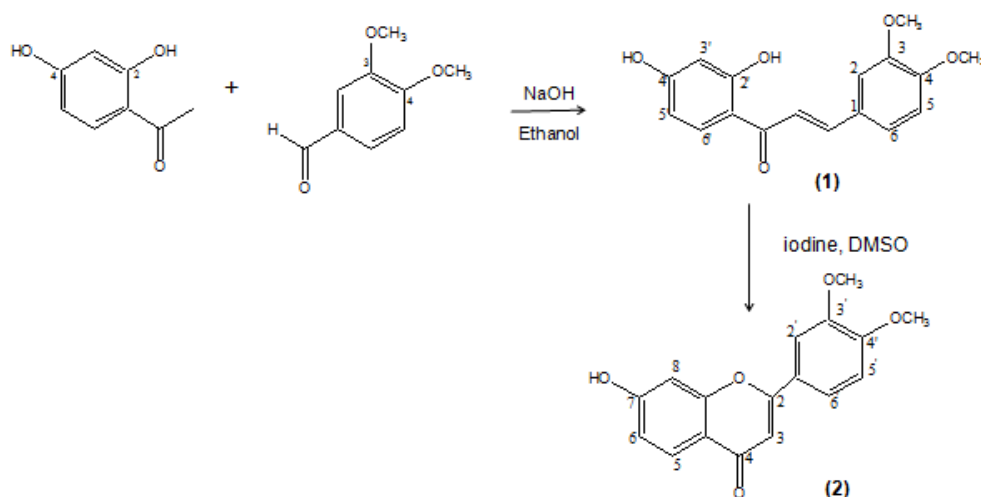


Fig 1. Synthesis of 7-hydroxy-3',4'-dimethoxyflavone

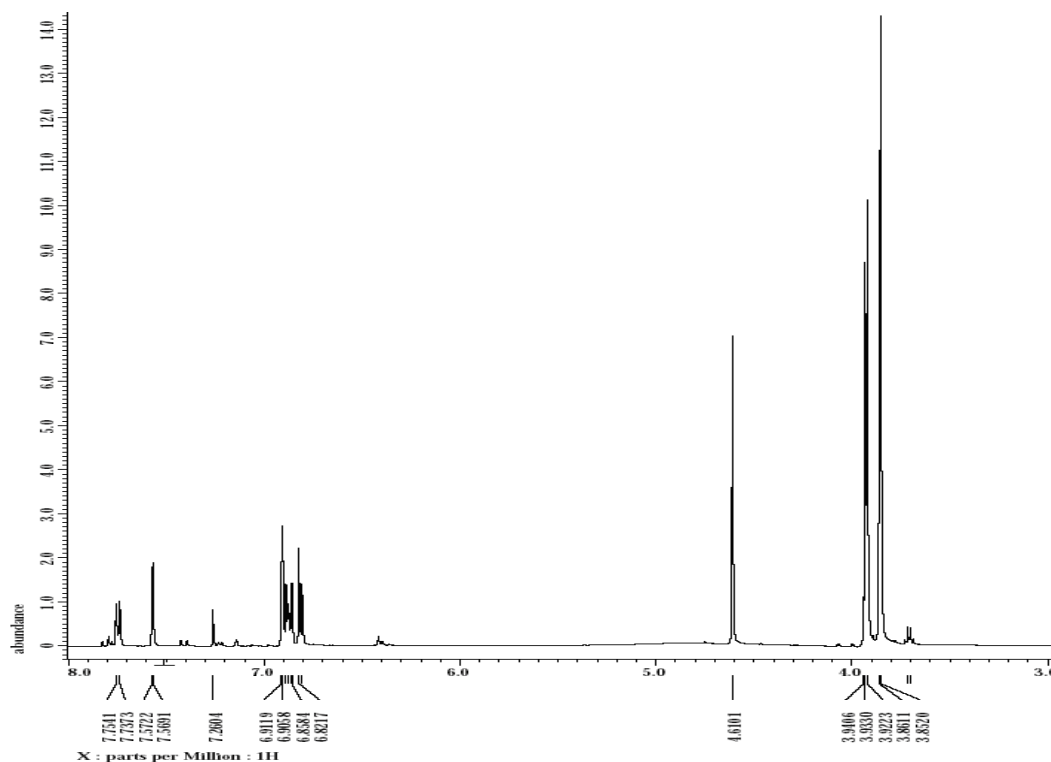


Fig 2. <sup>1</sup>H-NMR Spectrum of 2',4'-dihydroxy 3,4-dimethoxychalcone

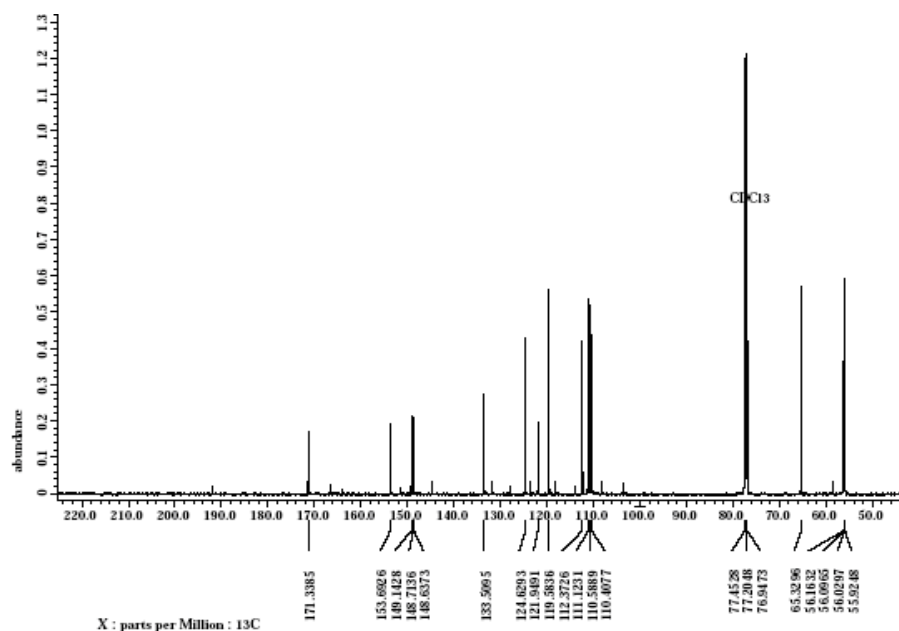
three measurements. % Inhibition =  $[A_{\text{Control}} - A_{\text{Test}}] / A_{\text{Control}} \times 100$ . [14]

## RESULT AND DISCUSSION

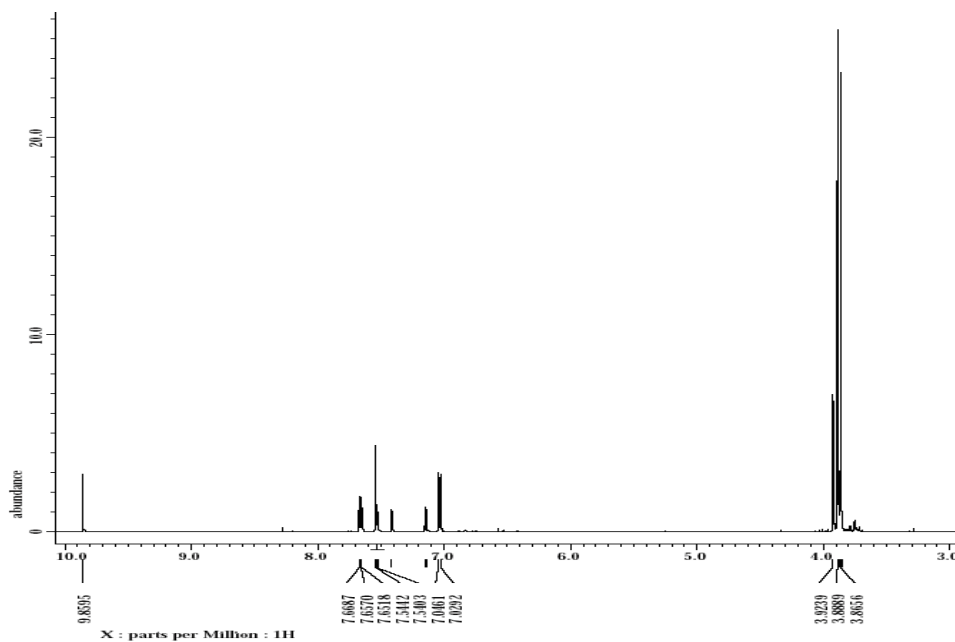
The synthetic approach to 7-hydroxy-3',4'-dimethoxyflavone (2) is as illustrated in Fig. 1. The Claisen-Schmidt condensation of 2,4-dihydroxyacetophenone with commercially available 3,4-dimethoxybenzaldehyde under basic condition (NaOH 50%) in ethanol proceeded smoothly to furnish 2',4'-

dihydroxy-3,4-dimethoxychalcone (1) (40%). The compound was obtained as yellow-needle crystals and it gave orange color with Mg/HCl. The structure of this compound was confirmed by spectral data.

Spectral data, UV-Vis (nm): 211, 231, 278, 310; IR (KBr,  $\text{cm}^{-1}$ ): 3410, 3086, 2939, 1674, 1589, 1512, 1465, 1265; <sup>1</sup>H NMR ( $\text{CDCl}_3$ ):  $\delta$  3.85 (3H, C-3-OCH<sub>3</sub>), 3.86 (3H, C-4-OCH<sub>3</sub>), 4.6 (OH), 7.5 (1H, H- $\alpha$ ),  $\delta$  7.7 (1H, H- $\beta$ ), 6.90 (1H, H-5), 6.91 (1H, H-2), 7.2 (1H, H-6); <sup>13</sup>C-NMR ( $\text{CDCl}_3$ ):  $\delta$  171 (C=O), 153.6 (C-4<sup>1</sup>), 149.1 (C-2<sup>1</sup>), 148.7 (C-4), 148.6 (C-3), 121.9 (C-1), 111.1 (C-1<sup>1</sup>),



**Fig 3.**  $^{13}\text{C}$ -NMR Spectrum of 2',4'-dihydroxy-3,4-dimethoxychalcone



**Fig 4.**  $^1\text{H}$ -NMR Spectrum of 7-hydroxy-3',4'-dimethoxyflavone

133.5 (C- $\beta$ ), 119.5 (C- $\alpha$ ), 124.6 (C-6 $^1$ ), 112.3 (C-6), 110.5 (C-5), 110.4 (C-2), 65.3 (C-5 $^1$ ), 56.1 (C-3 $^1$ ), 56.02 (CH $_3$ ).

The UV-Vis absorbance band of this compound ( $\lambda_{\text{max}}$  211, 231, 278, 310) suggested the presence of a chalcone skeleton. The IR absorption spectrum band at 3410  $\text{cm}^{-1}$  indicated that the presence of hydroxyl group, and a band at 1674  $\text{cm}^{-1}$  and 1589  $\text{cm}^{-1}$  showed the presence of a conjugated carbonyl group.

The  $^1\text{H}$ -NMR spectrum of compound (Fig. 2) explained the presence of two methoxyl groups in the B-ring at  $\delta$  3.85 and 3.86, integrating for 12 protons. The phenolic -OH signal was observed at  $\delta$  4.6. The olefinic proton of  $\alpha,\beta$ -unsaturated ketone were clearly observed at  $\delta$  7.5 (1H, H- $\alpha$ ) and  $\delta$  7.7 (1H, H- $\beta$ ) corresponding to H- $\alpha$  and H- $\beta$ . The three aromatic protons of the B-ring were observed at  $\delta$  6.90 (1H, H-5),  $\delta$  6.91 (1H, H-2),  $\delta$  7.2 (1H, H-6).

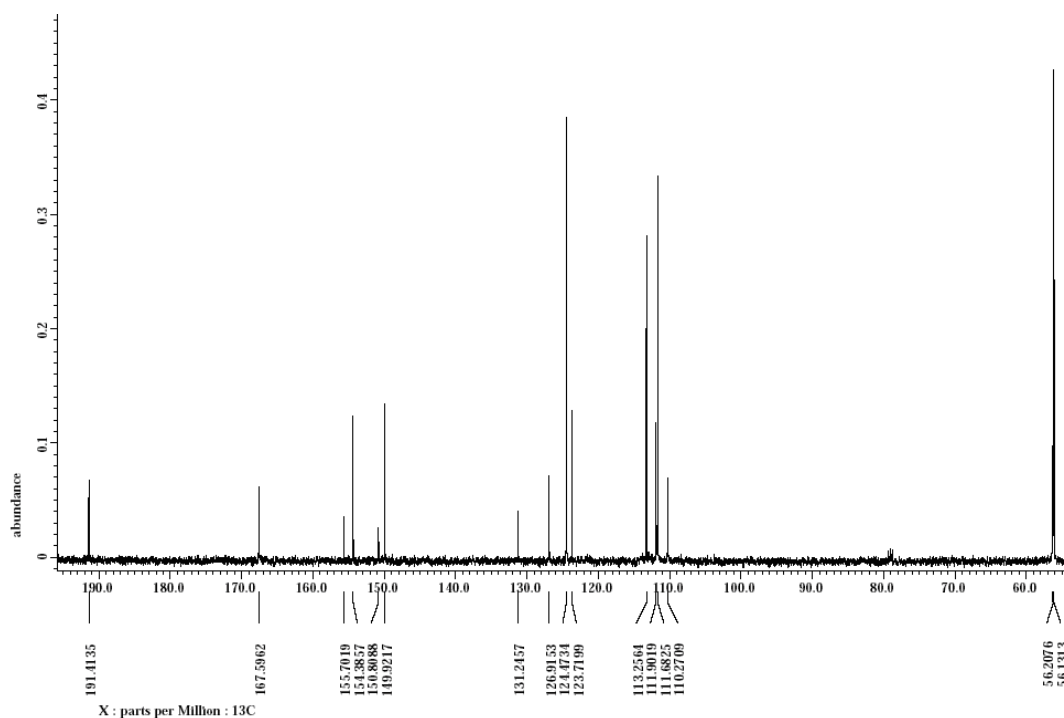


Fig 5.  $^{13}\text{C}$ -NMR Spectrum of 7-hydroxy-3',4'-dimethoxyflavone

The  $^{13}\text{C}$ -NMR spectrum of chalcone (Fig. 3) showed the presence of 17 different carbons. The signals for one carbonyl at  $\delta$  171, and signals for methyl carbons at  $\delta$  56.0. The spectrum indicated the presence six quaternary carbon at  $\delta$  153.6 (C-4<sup>1</sup>), 149.1 (C-2<sup>1</sup>), 148.7 (C-4), 148.6 (C-3), 121.9 (C-1), 111.1 (C-1<sup>1</sup>), and eight methine carbons at  $\delta$  133.5 (C- $\beta$ ), 119.5 (C- $\alpha$ ), 124.6 (C-6<sup>1</sup>), 112.3 (C-6), 110.5 (C-5), 110.4 (C-2), 65.3 (C-5<sup>1</sup>), 56.1 (C-3<sup>1</sup>), and two methoxy carbons at  $\delta$  56.02. Based on the above spectral evidences, it can be concluded that compound (**1**) characterized as 2',4'-dihydroxy-3,4-dimethoxychalcone.

The second sequence in synthesis required the cyclization of 2',4'-dihydroxy-3,4-dimethoxychalcone into 7-hydroxy-3',4'-dimethoxyflavone. Cyclization was carried out by using iodine in DMSO as catalyst. The compound was obtained as yellow-needle crystals and it gave orange color with Mg/HCl. The formation of flavone was supported by spectral data.

Spectral data; UV-Vis (nm): 261, 287 nm; IR (KBr,  $\text{cm}^{-1}$ ): 3402, 1674, 1589, 1512, 1265;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.02 (1H, s, H-3), 7.544 (H-2<sup>1</sup>), 7.65 (H-5<sup>1</sup>), 7.66 (H-6<sup>1</sup>), 7.04 (H-8), 7.540 (H-6), 9.8 (H-5);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ ):  $\delta$  191.4 (C=O), 56.2 ( $\text{CH}_3$ ), 167.59 (C-2), 155.7 (C-7), 154.3 (C-8a), 150.8 (C-3<sup>1</sup>), 149.9 (C-4<sup>1</sup>), 126.9 (C-1<sup>1</sup>), 123.7 (C-4a), 131.2 (C-5), 124.47 (C-6<sup>1</sup>), 113.2 (C-2<sup>1</sup>), 111.9 (C-5<sup>1</sup>), 111.6 (C-6), 110.27 (C-8), 56.34 (C-3), 56.2 (C3<sup>1</sup>-OCH<sub>3</sub>), 56.1 (C4<sup>1</sup>-OCH<sub>3</sub>).

The UV spectrum of the compound displayed absorption bands at 261 and 287 nm suggested the

presence of a flavone skeleton. The IR spectrum of compound displayed bands absorption for -OH ( $3402\text{ cm}^{-1}$ ). The absorption bands at  $1674\text{ cm}^{-1}$  as well as  $1589$  and  $1512\text{ cm}^{-1}$  indicating the presence of a conjugated carbonyl group and aromatic rings respectively. The presence of C-O is indicated by the absorption at  $1265\text{ cm}^{-1}$ . In the  $^1\text{H}$  NMR spectrum (Fig. 4), the absence of a phenolic proton signal at upfield region was noted; indicating that the oxidative cyclization of chalcone has took place. This is also supported by the presence of a characteristic singlet signal for a flavone at  $\delta$  7.02 (1H, s, H-3). The aromatic protons of B ring at  $\delta$  7.544 (H-2<sup>1</sup>), 7.65 (H-5<sup>1</sup>), and 7.66 (H-6<sup>1</sup>). Whereas the three aromatic proton of the ring A appeared at  $\delta$  7.04 (H-8), 7.540 (H-6) and 9.8 (H-5).

The  $^{13}\text{C}$ -NMR spectrum of flavone (Fig. 5) showed the presence of 17 different carbons. The signals for one carbonyl at  $\delta$  191.4, and signals for methyl carbons at  $\delta$  56.2. The spectrum indicated the presence seven quaternary carbon at  $\delta$  167.59 (C-2), 155.7 (C-7), 154.3 (C-8a), 150.8 (C-3<sup>1</sup>), 149.9 (C-4<sup>1</sup>), 126.9 (C-1<sup>1</sup>), 123.7 (C-4a) and seven methine carbons at  $\delta$  131.2 (C-5), 124.47 (C-6<sup>1</sup>), 113.2 (C-2<sup>1</sup>), 111.9 (C-5<sup>1</sup>), 111.6 (C-6), 110.27 (C-8), 56.34 (C-3), and two methoxy carbons overlapping at  $\delta$  56.1. Based on the above spectral evidences, it can be concluded that compound (**2**) characterized as 7-hydroxy-3',4'-dimethoxyflavone.

In this study, the antioxidant activities of the synthesized compounds were measured using DPPH method. The DPPH radical scavenging assay has been widely used to assess the antioxidant ability of compounds. This method is based on the ability of DPPH radical to react with hydrogen donor species such as phenolics and flavonoids present in the extract material. Upon receiving a proton from the donor species it loses its color and becomes yellow. As the concentration of phenolic compounds increases their DPPH radical scavenging activity also increases.

The free radical scavenging activity of compound was investigated with  $IC_{50}$  (the extract concentration inhibiting 50% of DPPH radicals). Both of compound 2',4'-dihydroxy-3,4-dimethoxychalcone and 7-hydroxy-3',4'-dimethoxyflavone tested showed low radical scavenging activity with  $IC_{50}$  more than 100  $\mu\text{g/mL}$ .

## CONCLUSION

7-hydroxy-3',4'-dimethoxyflavone has been synthesized by oxidative cyclization of 2',4'-dihydroxy-3,4-dimethoxychalcone. The synthesized compounds were characterized by UV-Vis, IR,  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectroscopy. The result obtained from this research confirmed that the product had been formed.  $IC_{50}$  (the extract concentration inhibiting 50% of DPPH radicals) values for this compound more than 100  $\mu\text{g/mL}$ , showed that this compound was not active as an antioxidant.

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