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Research Article

SYNTHESES AND ANTIOXIDANT ACTIVITIES OF SOME HYDROXY DIMETHOXY CHALCONE DERIVATIVES

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ABSTRACT

Four (4) hydroxy dimethoxy chalcones derivatives were synthesized by Claisen-Schmidt condensation of hydroxyacetophenone with 3,4-dimethoxybenzaldehyde using conventional method and grinding technique. The synthesized compounds were characterized by spectroscopy (IR, 1H-NMR and 13C-NMR). Synthesis of the hydroxy dimethoxy chalcone using grinding method is better than the conventional method. The synthesis using grinding method require a low concentration base, shorter reaction (15min) and higher yield (70-84%). The antioxidant activity of the compounds was determined by DPPH method, showed that 2',5'-dihydroxy-3,4-dimethoxy chalcone have the highest antioxidant activity.

Key word: hydroxy dimethoxy chalcone, Claisen-Schmidt condensation, grinding, DPPH method

INTRODUCTION

Hydroxy chalcone known as intermediates for the synthesis of a variety of flavonoids, such as flavones, isoflavones, flavanones and flavonols. This compound has a skeletal system of 1,3-diaryl-1-one, has pharmacological activity, such as anticancer (Patil, *et al.,* 2009), antimicrobial (Mandge, *et al,* 2007 and Prasad, *et al,* 2008), insecticides (Nalwar, *et al.,* 2009) and antioxidants (Belsare, *et al.,* 2011). Synthesis is the best alternative for obtaining chalcone, because stable chalcone difficult to obtain due to the existence of the plant enzyme chalcone synthetase (CSH) which converts chalcone into flavanones (Mandge, *et al.,* 2007).

Chalcone synthesis method most widely developed is Claisen-Schmidt condensation. Prasad *et al.* (2008) have synthesized chalcone derivative 12 by Claisen-Schmidt condensation of acetophenone derivatives and benzaldehyde derivatives using KOH as catalyst in ethanol. The study found that compounds with hydroxy and methoxy groups showed better antibacterial activity than chalcone without methoxy or hydroxy groups. Four chalcone derivative (4-dimethyl amino chalcone, 2-hydroxy-4-dimethyl amino chalcone, and 2-hydroxy chalcone) has been synthesized in this way using alkaline catalyst (KOH 60%) in ethanol (Mandge *et al.,* 2007).

In this study 4 hydroxy chalcone derivatives were synthesized by Claisen-Schmidt condensation from 3,4-dimethoxy-benzaldehyde using grinding and conventional method, then analyzed its antioxidant activity. Reaction in grinding technique are simple due to reactions are initiated by grinding, occurs through generation of local heat by grinding of crystals of substrate and reagent by mortar and pestle (Zangade, *et al.,* 2011). This method is used to develop chalcone synthesis method that is more friendly to the environment and high yielding.

Many studies have found that chacone exhibit various biological activities, such as antiviral, anti-inflammatory, and anticancer. Bioactivity is generally attributed to its antioxidant properties of chalcone, namely their ability to protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals, and hydroxyl radicals. These free radicals are involved in the process of a number of diseases, such as cancer, aging, atherosclerosis, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's), as well as hearing loss. Imbalance between antioxidants and reactive oxygen species in oxidative stress causes damage to cells (Warner, *et al.,* 2004.)

Antioxidants react with free radicals at a higher speed than the substrate. Free radicals can attack a variety of targets including lipids, fats and proteins, so it is believed that free radicals damage the organism, causing illness, aging and toxic (Wright, *et al.,* 2001). Hydroxy chalcone can capture free radicals directly. These compounds are oxidized by radicals, forming less reactive radicals. In other words, hydroxy chalcone stabilize reactive oxygen species by reacting with radicals. Hydroxy chalcone high reactivity causes radical becomes inactive (Narayana, *et al.,* 2001). For the purpose of the development of antioxidant agents, we needed to modify the structure or substitute suitable groups in the structure of hydroxy chalcone to improve antioxidant activities. Therefore, we synthesized a series of hydroxy-3,4-dimethoxy chalcone and examined their antioxidant activities.

MATERIAL AND METHODS

Synthesis of hydroxy dimethoxy chalcone using conventional method

A mixture of hydroxyacetophenone (0.01mole) and 3,4-dimethoxybenzaldehyde derivatives (0.01mole) were stirred in ethanol (15mL) and then an aqueous of sodium hydroxide 50% (12mL) were added. The reaction mixture was stirred at room temperature and left at room temperature for 24h, poured into iced water, acidified with cold HCl (10%), and extracted with ether (3x25mL). The organic layer was washed with water, dried by addition over anhydrous Na_2SO_4. The solvent was evaporated by rotary evaporator. Purification the compound was done by Column chromatography on a silica gel column (n-hexane:acetone, 7:3), recrystalized by ethanol and characterized by UV Vis, IR, ^1H NMR and ^{13}C NMR Spectroscopy. Four kinds of hydroxyl 3,4-dimethoxy chalcone derivatives were synthesized using base material 2-hydroxy acetophenone, 2,4-dihydroxy acetophenone, 2,5-dihydroxy acetophenone and 2,6-dihydroxy acetophenone.

Synthesis of hydroxy dimethoxy chalcone using grinding method

The hydroxyacetophenones (5mmol), 3,4-dimethoxybenzaldehyde (10mmol) and solid NaOH (20mmol) was ground with a mortar and pestle at room temperature for 15min. The obtained solid mixture was diluted with cold water, added cold HCl (10%) until pH 2-4, and extracted with ether. The ether layer was washed with water, dried by over anhydrous Na_2SO_4 and evaporated. Purification using by Column chromatography on a silica gel column (n-hexane:acetone, 6:4), followed by recrystalization. Identification of chalcone structure using by UV Vis, IR, NMR Spectroscopy.

Antioxidant activity of compounds was performed by DPPH method.

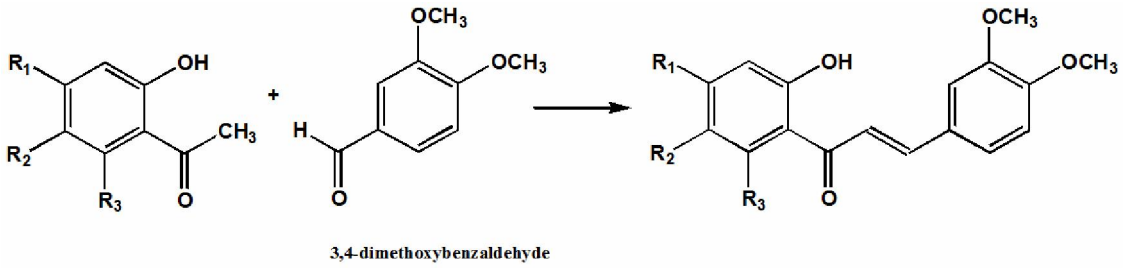
In vitro antioxidant activity of compounds synthesized done by DPPH method (Belsare *et al.,* 2010), a method used to determine the activity of free radicals capture. Samples were dissolved in methanol and prepared in various concentrations (0, 10, 30, 50 and 70ppm). Each solution is put into a test tube. Into each tube was added 500μL solution of 1mM DPPH in methanol, refined to 5.0mL, and then incubated at 37°C for 30min. The solution was measured at λ 515nm. Positive controls used vitamin C. Each measurement is done 3times. Capture of free radicals by DPPH was calculated with the equation:

Activity (% DPPH reduction) = [(A-Ax) / A] x 100%

where A = absorbance of DPPH solution in methanol, Ax = absorbance of DPPH solution with sample extracts. IC50 is defined as the sample concentration that showed 50% capture of radical activity, determined from the relationship graph with the concentration of the antioxidant activity.

RESULTS AND DISCUSSION

The Claisen-Schmidt condensation reaction is an important method for synthesis of chalcone. The synthesis of hydroxy dimethoxy chalcone is a single step method. The yield of each chalcone synthesized could be seen in Table I. Synthesis of the hydroxy dimethoxy chalcone using grinding method is better than the conventional method. The synthesis using grinding method require a low concentration base, shorter reaction (15min) and higher yield (70-84%).



2'-hydroxy-3,4-dimethoxy chalcone: $R_1=R_2=R_3=H$; 2',4'-dihydroxy-3,4-dimethoxy chalcone: $R_1=OH$, $R_2=R_3=H$; 2',5'-dihydroxy-3,4-dimethoxy chalcone: $R_2=OH$, $R_1=R_3=H$; 2',6'-dihydroxy-3,4-dimethoxy chalcone: $R_3=OH$, $R_1=R_2=H$

Figure 1. Scheme of synthesis hydroxyl dimethoxy chalcone derivatives.

Table I. Yield of derivatives of hydroxy chalcone synthesized

No	Compound	Yield (%)	
		conventional	grinding
1.	2'-hydroxy-3,4-dimethoxychalcone	75	-
2.	2',4'-dihydroxy-3,4-dimethoxychalcone	70	84
3.	2',5'-dihydroxy-3,4-dimethoxychalcone	45	73
4.	2',6'-dihydroxy-3,4-dimethoxychalcone	65	70

Table II. IC 50 of derivatives of hydroxy chalcone synthesized

No	Compound	IC 50 (µg/mL)
1.	2'-hydroxy-3,4-dimethoxychalcone	975
2.	2',4'-dihydroxy-3,4-dimethoxychalcone	1402.9
3.	2',5'-dihydroxy-3,4-dimethoxychalcone	7.34
4.	2',6'-dihydroxy-3,4-dimethoxychalcone	1058

The structure of the synthesized compounds was confirmed by IR, ^{1}H-NMR and ^{13}C-NMR.

2'-hydroxy-3,4-dimethoxy chalcone

The IR peak at 3549cm^{-1} suggesting the presence of -OH group The IR peak at 1627cm^{-1} suggesting the presence of C=O (Str) group. The IR peak at 3078cm^{-1} indicates the presence of C-H aromatic. The IR peak at 2931cm^{-1} indicates the presence of C-H aliphatic. The IR peak at 1026cm^{-1} indicates the presence of C-O. The ^{1}H-NMR spectrum of 2-hydroxy-3,4-dimethoxy chalcone (Figure 2) displayed multiplet due to overlapping of signal for two methoxyl groups at δ 3.91 integrated for 6 protons. The phenolic –OH signal was observed at δ 12.89. The three aromatic proton of the ring B were observed at δ 6.94 (1H, C-2); δ 7.0 (1H, C-6); and 7.2 (1H, C-5). Four aromatic proton of the ring A were observed at δ 7.4 (1H, C-3'); 7.5 (1H, C-5'); 7.90 (1H, C-4'); and 7.92 (1H, C-6').

The ^{13}C-NMR spectrum of 2-hydroxy-3,4-dimethoxy chalcone (Figure 3) indicated the presence of 16 signals attributed to 17 different carbons. The signal for methyl carbons were overlapping with each other at 56,01. The spectrum indicated the presence five quarternary carbon at δ δ 163.5 (C-2^1), 151.8 (C-3), 149.3 (C-4), 127.5 (C-1), dan 120,1 (C-1'), dan 9 karbon metin pada δ 145.6 (C-4'), 136.1 (C-α), 129.5 (C-6'), 123.5 (C-β), 118.7

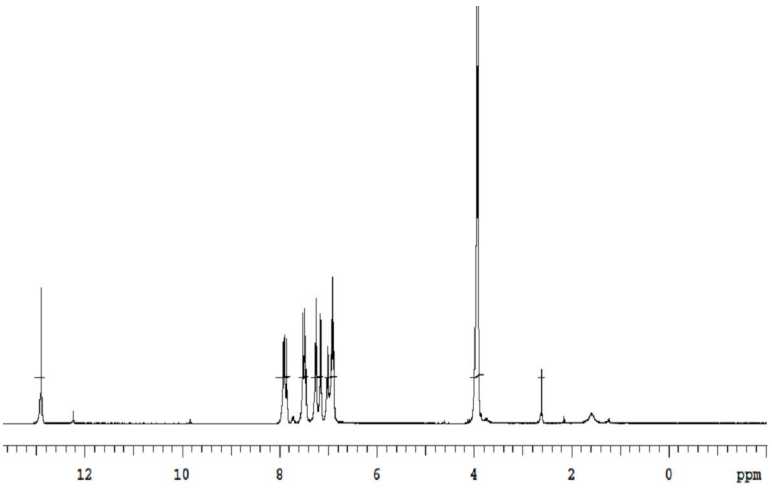


Figure 2. ^{1}H NMR Spectrum of 2'-hydroxy-3,4-dimethoxy chalcone

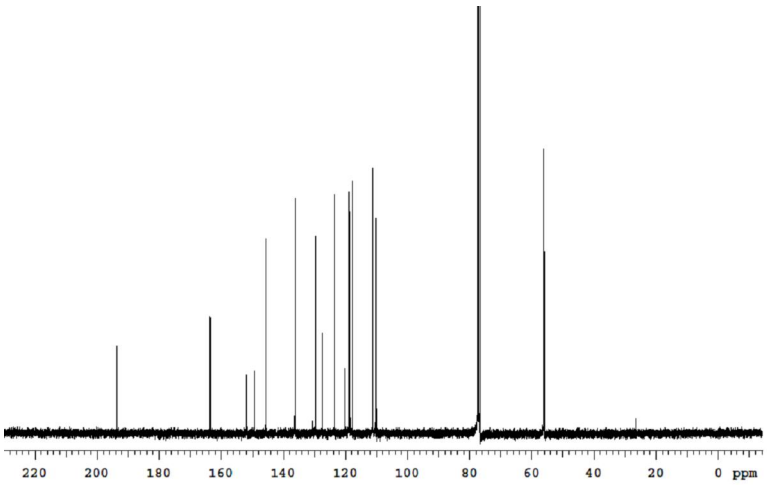


Figure 3. ^{13}C NMR Spectrum of 2'-hydroxy-3,4-dimethoxy chalcone

(C-6), 118.5 (C-5'), 117 (C-3'), 111 (C-2), 110 (C-5), and 9 methine carbons at δ 145.6 (C-4'), 136.1 (C-α), 129.5 (C-6'), 123.5 (C-β), 118.7 (C-6), 118.5 (C-5'), 117 (C-3'), 111 (C-2), 110 (C-5). Based on the above spectral evidences, it can be concluded that compound **(1)** characterized as 2'-hydroxy-3,4-dimethoxy chalcone.

2',4'-dihydroxy-3,4-dimethoxy chalcone

The IR absorption spectrum band at 3410 cm^{-1} indicated that the presence of hydroxyl group, and a band at 1674cm^{-1} and 1589cm^{-1} showed the presence of a conjugated carbonyl group. The IR peak at 3086cm^{-1} indicates the presence of C-H aromatic. The IR peak at 2939cm^{-1} indicates the presence of C-H aliphatic. The IR peak at 1026cm^{-1} indicates the presence of C-O.

The ^{1}H-NMR spectrum of compound (Figure 4) displayed the presence of two methoxyl groups in the B ring at δ 3.85 and 3.86, integrating for 6 protons. The phenolic –OH signal was observed at δ 4.6. The olefinic proton of α,β-unsaturated ketone were clearly observed at δ 7.5 (1H, H-α) and

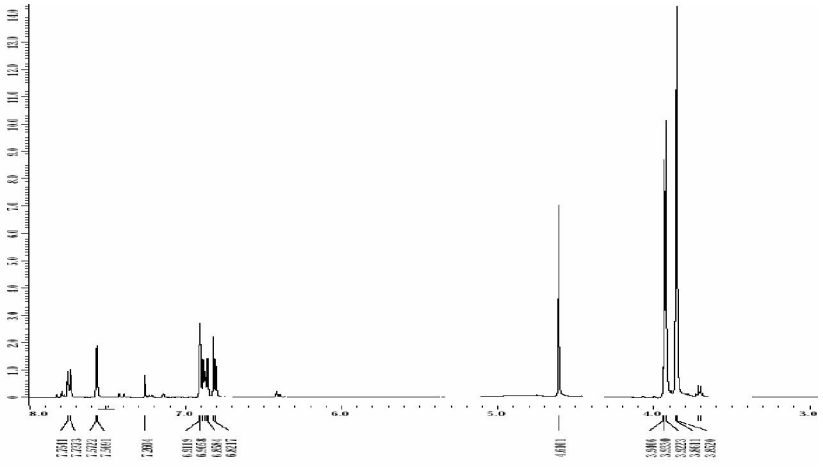


Figure 4. ^{1}H NMR Spectrum of 2',4'-dihydroxy 3,4-dimethoxy chalcone

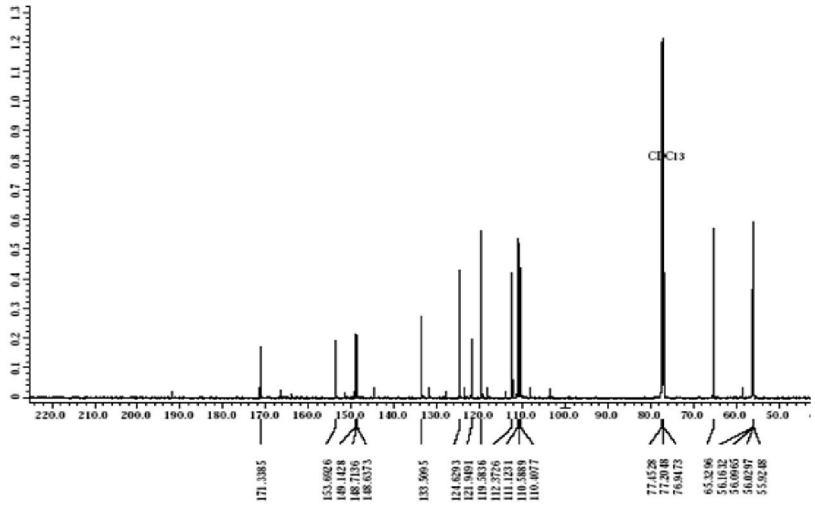


Figure 5. ^{13}C NMR Spectrum of 2',4'-dihydroxy-3,4-dimethoxychalcone

δ 7.7 (1H, H-β) corrresponding to H-α and H-β. The three aromatic protons of the B-ring were observed at δ 6.90 (1H, H-5), δ 6.91 (1H, H-2), δ 7.2 (1H, H-6).

The ^{13}C-NMR spectrum of chalcone (Figure 5) showed the presence of 17 different carbons. The signals for one carbonyl at δ 171, and signals for methyl carbons at δ 56,0. The spectrum indicated the presence six quarternary carbon at δ 153.6 (C-4^{1}), 149.1 (C-2^{1}), 148.7 (C-4), 148.6 (C-3), 121.9 (C-1), 111.1 (C-1^{1}), and eight methine carbons at δ 133.5 (C-β), 119.5 (C-α), 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}), and two methoxy carbons at δ 56,02. Based on the above spectral evidences, it can be concluded that compound **(2)** characterized as 2',4'-dihydroxy-3,4-dimethoxy chalcone.

2',5'-dihydroxy-3,4-dimethoxy chalcone

The IR spectrum has the IR absorptions characteristics of hydroxy (3410cm^{-1}), aromatic C-H (3062cm^{-1}), aliphatic C-H (2939cm^{-1}), carbonyl C=O (1674cm^{-1}), alkenes (1465cm^{-1}), and C-O (1026cm^{-1}), functionalities. The ^{1}H-NMR spectrum of 2',5'-dihydroxy-3,4-dimethoxychalcone (Figure 6) explained the presence of two methoxyl groups in the B ring at δ 3.86 and 3.87, integrating for 12 protons.

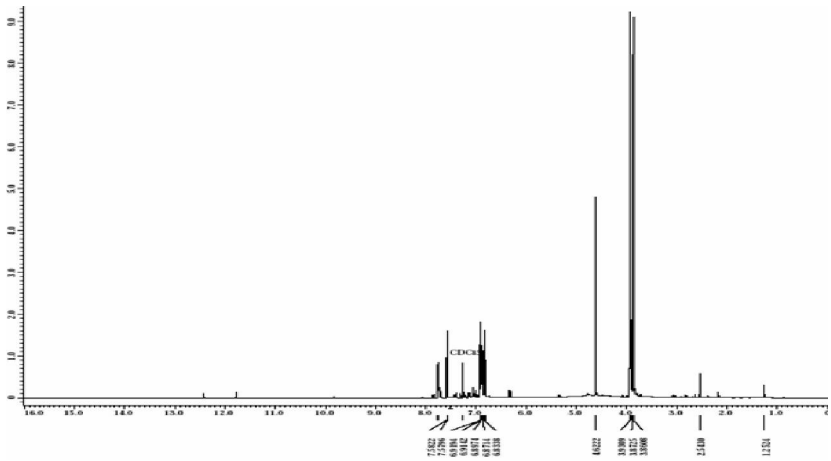


Figure 6. ^{1}H-NMR spectrum of 2',5'-dihydroxy-3,4-dimethoxy chalcone

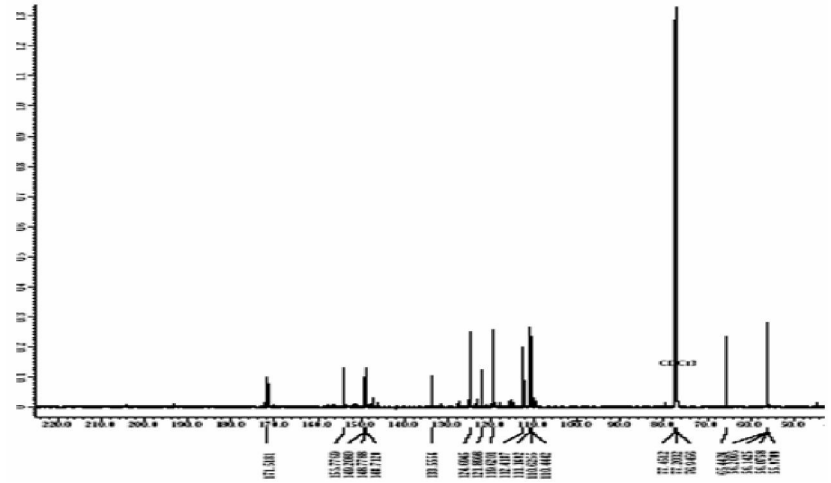


Figure 7. ^{13}C-NMR spectrum of 2',5'-dihydroxy-3,4-dimethoxy chalcone

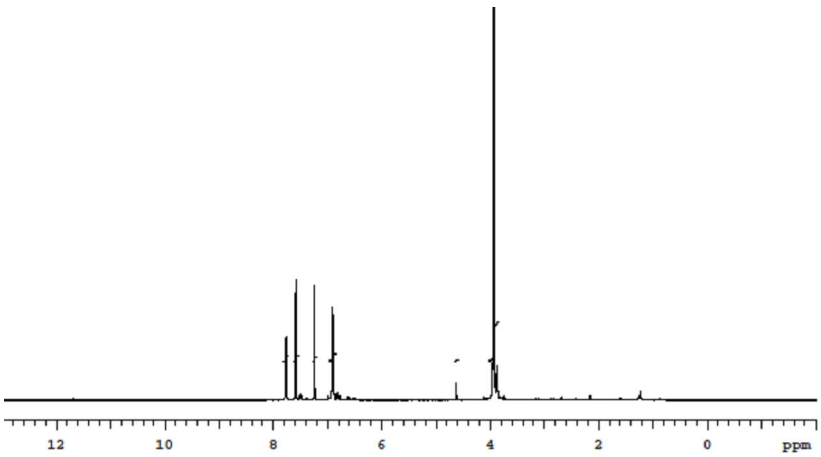


Figure 8. ^{1}H-NMR spectrum of 2',6'-dihydroxy-3,4-dimethoxy chalcone

The olefinic proton of α,β-unsaturated ketone were clearly observed at δ 6.91(1H, H-α) and δ 6.92 (1H, H-β) corrresponding to H-α and H-β. The ^{13}C-NMR spectrum of chalcone (Figure 7) showed the presence of 17 carbon with one carbonyl at δ 171.5, δ 153.7, 149.2, 148.77, 148.71, 133.5, 124.6, 121.8, 119.6, 112.4, 111.1, 110.6, 110.4, 65.4, 56.2, 56.1. The signals for methyl carbons were overlapping with each other at δ 56,0 confirmed the presence of two methyl carbons. Based on the above spectral evidences, it can be concluded that compound **(3)** characterized as 2',5'-dihydroxy-3,4-dimethoxy chalcone.

2',6'-dihydroxy-3,4-dimethoxy chalcone

The IR spectrum of the compound displayed streching bands for hydroxyl group at 3425cm^{-1} and carbonyl group at 1674cm^{-1}. Its IR spectrum also showed bands for C-H aliphatic at 2939cm^{-1}, C=C group at 1427cm^{-1}, and C-0 streching at 1026cm^{-1}. The ^{1}H-NMR spectrum of the chalcone (Figure 8) explained the presence of two methoxyl groups in the B ring at δ 3.8 and 3.9 integrating for 6 protons. The olefinic proton of α,β-unsaturated ketone were clearly observed at δ 7.77 (1H, H-α) and δ 7.75 (1H, H-β) corrresponding to H-α and H-β. Based on the above spectral evidences, it can be concluded that synthesized compound **(4)** characterized as 2',6'-dihydroxy-3,4-dimethoxy chalcone.

Antioxidant activity

In vitro antioxidant activity of the compounds synthesized was measured by DPPH methods, to determine the free radical scavenging activity. The DPPH radical scavenging activities of synthesized compounds were comparable to the activity of ascorbic acid. Ascorbic acid as a positive control gave IC50 4.66μg/mL. The antioxidant activity data of the hydroxy chalcone (Table II) indicate that 2',5'-dihydroxy-3,4-dimetoksikalkon most active as a free-radical scavenging.

CONCLUSION

It could be concluded that four of hydroxy dimethoxy chalcone compounds (ie 2'-hydroxy-3,4-dimethoxy chalcone, 2',4'-dihydroxy-3,4-dimethoxy chalcone, 2',5'-dihydroxy-3,4-dimethoxy chalcone, 2',6'-dihydroxy-3,4-dimethoxy chalcone) can be synthesized from 3,4-dimethoxybenzaldehyde by Claisen-Schmidt condensation. The hydroxy dimethoxy chalcone synthesized were characterized by spectroscopic (IR, ^{1}H-NMR and ^{13}C-NMR), indicating that the synthesis product has been formed. Synthesis of the hydroxy dimethoxy chalcone using grinding method is better than the conventional method. Measurement of antioxidant activity in vitro by DPPH method showed that only compound 2',5'-dihydroxy-3,4-dimethoxy chalcone active as an antioxidant.

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