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Synthesis and Toxicity Test of 2'-Hydroxy-5'-Chloro-3,4-Dymethoxychalcone

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Abstract. This study was aimed to synthesis the chalcone derivative and tested its toxicity. Synthesis of 2'-hydroxy-5'-chloro-3,4-dimethoxychalcone carried out through Claisen-Schmidt condensation at room temperature. The toxicity test prepared by *Brine Shrimp Lethality Test* (BSLT) to *Artemia Salina* L. Analysis retrosynthetic gives the 2-hydroxy-5-chloro acetophenone and 3,4-dimethoxy benzaldehyde as starting materials. The desire chalcone was confirmed by ^{1}H and ^{13}C NMR, also mass spectroscopy. The chalcone obtained in 83.27% and exhibited LC_{50} value in 974.99 µg/mL.

INTRODUCTION

Chalcone family have much interest not only from the synthetic and biosynthetic perspective but also due to their broad interesting biological activities. Chalcone an intermediate of flavonoid synthetic pathway. They exhibited biological and pharmacological activities such as anti inflammatory, antioxidant, antidiabetic, antitumor, anticancer and antibacterial [1]-[6]. Chalcones widely distributed in vegetables, fruits, tea, and other plants [7]. Chalcones structure are have a common scaffold of 1,3-diaryl-2-propen-1-one, that exist as *trans* and *cis* isomers with the trans is more thermodynamically stable [8]. Generally, synthesis chalcones conduct via conventional Claisen-Schmidt condensation reaction of aryl ketones and aromatic aldehyde. This reaction performs in the presence of alkaline and acid catalyst in liquid solvent as condensing agents. In previous studies, synthesis of chalcones derivative of 2',4'-dihydroxy-4-methoxychalcone via Claisen-Schmidt condensation afforded a moderate yield in 60,74% [9]. In addition, study of structure-activity relationship of methoxy group in aromatic ring B chalcone showed that the methoxy group at C4 position in ring B gave a good cytotoxic activity with IC_{50} 31.75 µg/mL, but increasing the number of methoxy group in ring B reduced the cytotoxic activity [2]. The halogenated chalcones were also have an important role for biologically activity of chalcones [10]. An 2',4'-dichloro-4-hydroxy-3-methoxychalcone has been synthesized and showed good toxicity and antioxidant activity with IC_{50} 26.10 µg/mL and LC_{50} 20.04 µg/mL, respectively [11]. In this study, we synthesized the chalcone that binds methoxy group at C4 position in Ring Band halogenated of Ring A with chloride, due to their potential to increase the cytotoxic activity of chalcone.

EXPERIMENT

Materials and Instrumentation of Research

The synthesis materials were used 2-hydroxy-5-chloroacetophenone, 3,4-dimethoxybenzaldehyde, sodium hydroxide (NaOH), methanol, ethanol, n-hexane, ethyl acetate, hydrogen chloride (HCl), and thin layer chromatography (TLC) plate (pa. Merck). Toxicicty test was used shrimp larvae of *Artemia salina* L, seawater,

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tween 80, and aquadest. The chalcone characterized by NMR JEOL JNMECA 500 MHz and MS QP2010S Shimadzu.

Synthesis of 2'-hydroxy-5'-chloro-3,4-dimethoxychalcone

The chalcone prepared by 2-hydroxy-5-chloroacetophenone (5 mmol) in methanol (10 mL) and stirred for a few minutes in room temperature till the compound completely dissolved (compound 1). Further step, 40% of NaOH (5 mL) added to compound 1, dropped it wisely and stirred. After that, the 3,4-dimethoxy benzaldehyde (5 mmol) in methanol (10 mL) added to mixture and stirred for 24 h.The reaction controlled by TLC with n-hexane: ethyl acetate (9:1) as eluens. The mixture was poured into ice-cold water and followed by neutralization with 10% of HCl till the pH 7. The yellow precipitate filtered off, washed and dried to give a desire chalcone.

Toxicity Test

The brine shrimp lethality test (BSLT) begins by incubating brine shrimp (A. salina) egg in a hatchery containing aerated seawater for 48 h. The egg will begin to hatch and being larvae that call as nauplii will actively to move and they were ready for BSLT testing. Ten of *A. salina* nauplii were fed into a test tube containing sample solution with various concentration 1000 µg/mL; 800 µg/mL; 600 µg/mL; 400 µg/mL; 200 µg/mL and added with seawater until the calibration limit of 10 mL. Observations were made 24 h after nauplii fed in the test tube [12]. The number of mortality larvaerecorded at each concentration including control. The experiment performed with three replications (triplet). The mortal shrimp larvae was calculatedby LC_{50} value using the Red and Muench analysis method.

RESULTS AND DISCUSSION

Synthesis of 2'-hydroxy-5'-chloro-3,4-dymethoxychalcone

The desire chalcone of 2'-hydroxy-5'-chloro-3,4-dymethoxychalcone characterized by ^{1}HNMR (Figure 1 and 2).

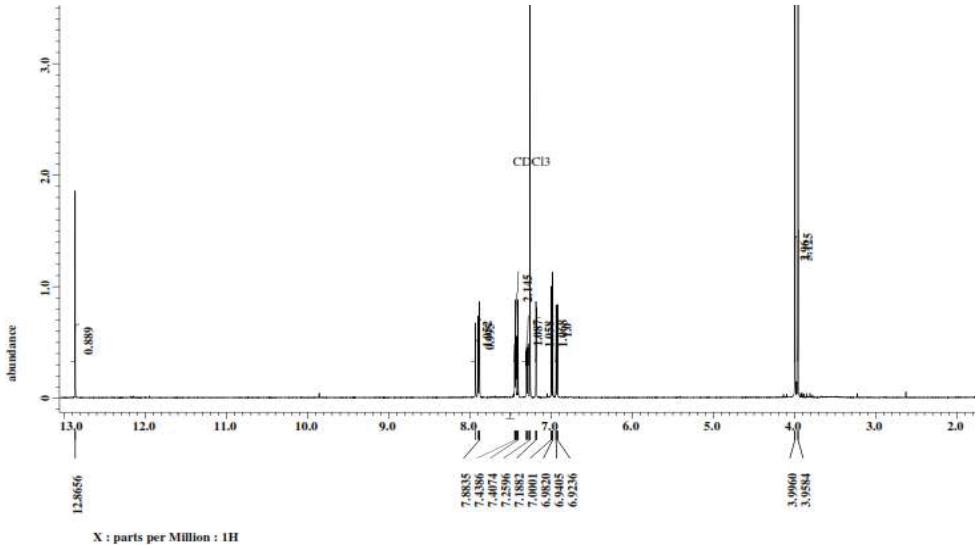

FIGURE 1. Spectrum^{1}H NMR of 2'-hydroxy-5'-chloro-3,4-dymethoxychalcone

According to ^{1}H and ^{13}C NMR spectrum, an2'-hydroxy-5'-chloro-3,4-dymethoxychalcoe was successfully synthesized in 83.27% yield with solid yellow. The chalcone controlled by TLC with eluent n-hexane:ethyl acetate (9:1), and resulted Rf value of 0.43. The chalcone also tested using flavonoids qualitative assay and gave a positive result. The ^{1}H NMR spectrum (500 MHz, $CDCl_3$, ppm): δ_H3.95 (3H, s, C3-OCH_3) ; 3.99 (3H, s, C4-OCH_3) ; 6.93 (1H, d, J = 8.4 Hz, H5) ; 6.99 (1H, d, J = 9 Hz, H3') ; 7.18 (1H, d, J = 1.9 Hz, H2) ; 7.29 (1H, dd, J = 8.4 ; 1.9 Hz, H6) ; 7.42 (1H, d, J = 15.6 Hz, Hα) ; 7.43 (1H, dd, J = 8.4 ;

2.6 Hz, H4') ; 7.48 (1H, d, *J* = 2,6 Hz, H6') ; 7.91 (1H, d, *J* = 14.9 Hz, Hβ) ; 12.8 (1H, H-OH). ^{13}C NMR spectrum ($CDCl_3$, ppm): δ_C 56.23 (C3-OCH_3); 56.27 (C4-OCH_3); 110.4 (C2); 111.3 (C5) ; 117.1 (C3') ; 120.3 (C6) ; 120.8(C-α) ; 123.5 (C1') ; 124.2 (C5') ; 127.4 (C1) ; 128.8 (C6') ; 136.1 (C4') ; 146.9 (C-β) ; 149.5 (C4) ; 152.2 (C3) ; 162.1 (C2') ; 192.7 (C=O).

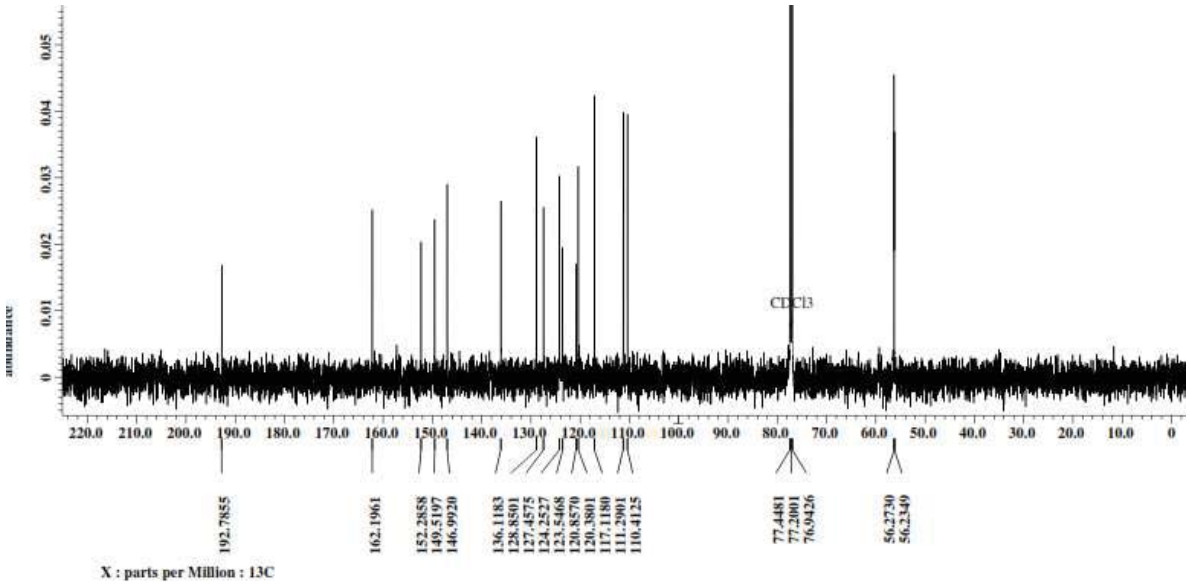

FIGURE 2. Spectrum ^{13}C NMR of 2'-hydroxy-5'-chloro-3,4-dymethoxychalcone

Synthesis of 2'-hydroxy-5'-chloro-3,4-dymethoxychalcone carried out by Claisen-Schmidt condensation reaction (Figure 3). The starting materials were used 2-hydroxy-5-chloroacetophenone and 3.4-dimethoxy benzaldehyde with ratio 1:1. Claisen-Schmidt condensation reaction run in alkaline conditions using NaOH. The first step reaction was nucleophilic addition reaction to form carbanion or enolate of 2-hydroxy-5-chloroacetophenone, followed with dehydration to form α,β-unsaturated ketone.Based on the ^{1}H NMR spectrum exhibited the 11 different protons (non-equivalent). Chemical shift in region 7.42 and 7.91 ppm with coupling constant *J*=15,25 Hz and type of peak doublet indicated the chalcone was formed. Those showed the Hα and Hβ which have the *trans* configuration. Singlet peak in δ3.95 ppm was proton of -OCH_3 that binds to C4. The proton with integration one proton in δ6.93 ppm (H5, *J* = 8.4 Hz); 7.18 ppm (H2, *J* = 1.9 Hz) and 7.29 ppm (H6, *J* = 8.4; 1.9 Hz) were aromatic proton of ring B. Those protons integrated each other and confirmed by the same of coupling constant value. However, the protons in δ= 6.99 ppm (H3', *J* = 9 Hz); 7.43 ppm (H2, *J* = 8.4; 2.6 Hz) and 7.48 ppm (H6, *J* = 2.6 Hz) were protons of aromatic ring A. Proton of OH typically appeared in δ 12.8 ppm.The spectra of ^{13}C NMR showed the 17 carbons. Absorption of C=O carbonyl appeared in δ 192.7 ppm and δ 56.23 and 56.27 for C-O methoxy. The six carbons quaternary were confirmed in δ 123.5 (C1'); 124.2 (C5'); 127.4 (C1); 149.5 (C4); 152.2 (C3); 162,1 (C2') and eight of methylene carbon were confirmed in δ 110.4 (C2); 111.3 (C5); 117.1 (C3'); 120.3 (C6); 120.8(C-α); 128.8 (C6'); 136.1 (C4'); 146.9 (C-β). The compound was also analyzed by Mass Spectroscopy; the result showed that molecular weight m/z of MS similar tothe calculating MW of 318 and molecular formula of $C_{17}H_{15}O_4Cl$. These data were confirmed that 2'-hydroxy-5'-chloro-3.4-dymethoxychalcone successfully synthesized

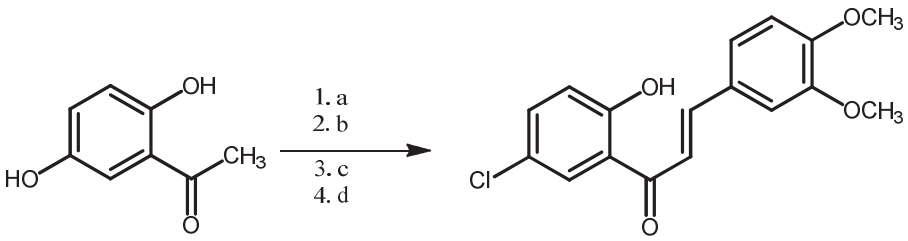

FIGURE 3. Synthesis of 2'-hydroxy-5'-chloro-3,4-dymethoxychalcone. Condition: a.methanol 10 mL; b. NaOH 40% 5 mL; c. 5 mmol of 3,4-dimethoxy benzaldehyde in methanol, stirred 24h; d. HCl 10%.

Toxicity Test of 2'-hydroxy-5'-chloro-3,4-dymethoxychalcone

The toxicity test of of 2'-hydroxy-5'-chloro-3,4-dymethoxychalcone carried out by BSLT method and Reed and Muench for data analyzing. The toxicity test gave LC_{50} value in 974.99 µg/mL, which is less toxic and not potent as cancer agent candidate [13]. Substituents can affect biological activity of chalcone. The carbonyl saturated system of Cα and Cβ have an important role for biologically activity of chalcone. A 2'-hydroxy-5'-chloro-3,4-dymethoxychalcone, which having two methoxy groups in Ring B and chloride in Ring A. These substituents affected the toxicity due to the slight positive mesomeric effect of halogen in para position at ring A. Halogen was electron withdrawing group, it reduced the distribution of electron to ethylene group. Similar to previous study, the chalcone derivative from methoxy benzaldehyde with halogen bind at C4 and para position of aromatic ketones (4-flouroacetophenone, 4-chloroacetophenone, and 4-bromoacetophenone) gave LC_{50} value higher than 200 ppm or less toxicity[14]. The previous study also compared the effect of number methoxy group in chalcone and the result showed the more of methoxy group in ring B chalcone gave less toxicity value against the HeLa cell [2].

CONCLUSION

The2'-hydroxy-5'-chloro-3.4-dymethoxychalcone has successfully synthesized via Claisen-Schmidt condensation with high yield of 83.27%, and it has less toxicity against the *Artemia salina* L.

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