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Synthesis and Biological Activity of Chlorochalcone Derivative

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Abstract. Chalcones is a compound that has many biological activities, but found in small amounts in plants. The synthesis of chalcones derived compounds was performed to obtain large amounts of chalcones compound in a relatively short time. In this research, derivative chalcone of 2'-hydroxy-5'-chloro-4-methoxychalcone was successfully synthesized for 72 hours in alkaline conditions at room temperature and obtained a yield of 60.06%. These compounds have been characterized using IR, MS, ^{1}H-NMR and ^{13}C-NMR. The result of bioactivity test by Brine Shrimp Lethality Test (BSLT) method showed that the compound had LC_{50} value 75.07 ppm and also antioxidant activity test by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method showed IC_{50} value of 45.99 ppm.

INTRODUCTION

Chalcone (1,3-diphenyl-2-propen-1-on) is a very important compound in nature. Chalcone has two aromatic rings (A and B) and one α, β unsaturated carbon atom. The chalcone compounds found in plants are the precursors of flavonoids[1]. The double bond between two aryl groups on chalcone structure plays an important role in the activity of the chalcon [2]. Several types of chalcone compounds exhibit biological activity such as antimalarial, anti-tuberculosis, anti-inflammatory, cytotoxic, antioxidant, analgesic, antiviral and antimicrobial [3].

Chalcone is very difficult to isolate from plants because of the enzyme chalcone synthetase (CSH) which easily converts chalcone into flavonoids. In addition, chalcone compounds are only found in a few plant groups and very small amounts. This is an obstacle for the development of chalcone compounds. Given the large number of balconies required and varying structures, it is necessary to make efforts to obtain balconies synthesis. Making balcony by means of this synthesis is advantageous, both in terms of cost and relatively short time [4]. Halogen-substituted chalcone compounds are difficult to find in nature. So it is necessary to develop the synthesis of chalcone compounds containing halogen groups. One way is to make chalcone which has a chloro group on one of its aromatic rings [5].

EXPERIMENT

Materials and Instrument

Nuclear magnetic resonance (NMR) spectra were performed using JEOL 400 MHz spectrometer, with tetramethylsilane as internal standard for ^{1}H, ^{13}C NMR using $CDCl_3$ solvent.Mass spectrometry spectra were recorded on QP 2010S Shimadzu and infrared spectra were recorded on FTIR Shimadzu Prestige 21. Materials 5-chloro-2-hydroxyacetophenone, 4-methoxybenzaldehide, sodium hydroxide, DPPH, hydrocloride acid and methanol.

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Synthesis of Chalcone

A total of 5 mmol 5-chloro-2-hydroxyacetophenone and5mmol 4-methoxybenzaldehyde were dissolved in 20 mL methanol. The solution was stirred at 25°C until dissolved and 5 mL 40% (w/v) NaOH was added, then stirred for 48 hours. Add 10% HCl slowly to form a crystalline solid. The addition of 10% HCl was stopped when the solution reached pH 7. Then the solid was filtered and washed with distilled water until the filtrate reached pH 7. The resulting product was dried in a desiccator for 24 hours. Compounds were purified using the column chromatography method and their structure was analyzed using infrared spectroscopy, mass spectroscopy, ^{1}H-NMR and ^{13}C-NMR.

Toxicity Test

The synthesized compound of 100 mg is dissolved in 1000 mL of seawater (1000 ppm). Then 10 mL of calibrated vials were prepared and 10 shrimp larvae were added to each vial. After that, 200 µL, 400 µL, 600 µL, 800 µL and 1000 µL of stock solution were added to each vial which had been calibrated 10 ml, made for five replications. Then sea water is added to the mark of the vial boundary and different concentration variations are obtained, namely 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. The level of compound toxicity is measured by counting the number of shrimp larvae that are still alive in 24 hour intervals. The data obtained were analyzed using Reed and Muench to determine the LC_{50} value of the synthesized compound.

Antioxidant Activity Test

A total of 100 mg of the synthesized compound were dissolved in 100 mL of methanol (Merck) (1000 ppm). Then 5 series of concentrations were made, namely 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. Each concentration series solution was inserted as much as 2 mL into a closed test tube made for 3 replications. Next, a 40 ppm DPPH stock solution was made by weighing 4 mg of DPPH dissolved with methanol in a 100 mL dark volumetric flask, the DPPH stock solution was then put 2 mL into a test tube containing a concentration series solution, left for 30 minutes and measured its absorbance using UV-VIS Spectrophotometry at 516 nm. Then the% antioxidant activity was calculated and the IC_{50} value was determined.

RESULTS AND DISCUSSION

Synthesis of Chlorochalcone

Chlorochalcone synthesis was initiated by dissolving the 5-chloro-2-hydroxyacetophenone and 4-methoxybenzaldehyde compounds first with absolute methanol. Then the base NaOH was added as a catalyst and stirred using a magnetic stirrer for 48 hours.The 2-hydroxy-5-chloroacetophenone compound which loses one H atom because it reacts with the -OH ion from the base NaOH will form an enolate ion. NaOH acts as a catalyst because it accelerates the reaction of the formation of chalcone compounds by forming enolate ions. The enolate ion formed then undergoes an addition reaction with the carbonyl in the 4-methoxybenzaldehyde compound and ends with the release of water molecules, thus forming the target compound. Purification of target compounds was carried out using column chromatography methods with n-hexane and ethyl acetate (11:1) eluents. The yield of the synthesized compound was 84.54%.

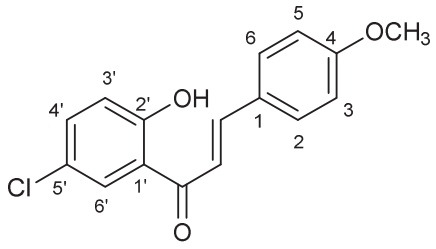

FIGURE 1. 2'-hydroxy-5'-chloro-4-methoxychalcone

Determination of the structure of the target compound was carried out using infrared spectroscopy, mass spectroscopy, ^{1}H-NMR and ^{13}C-NMR methods. IR spectroscopy is used to identify the types of bonds or functional groups present in the synthesized compound. The synthesized compound has an O-H bond which is located at the wave number 3263 cm^{-1}. Thecarbonyl bond is usually at 1600-1800 cm^{-1}, the results of the IR spectra data show one peak at 1604 cm^{-1} which is the carbonyl bond. The aromatic C=C bond is generally found at 1450-1600 cm^{-1}, the results of the IR spectra also show that there is a peak at ~~the~~ wave number 1558 which is C=C aromatic. The trans C=C bond is an important marker for chalcone compounds. The C=C trans bond is at wave number 675-995 cm^{-1}. The molecular weight of a compound is indicated by a mass spectrum using mass spectroscopy with a molecular peak of m/z 288.

TABLE 1. Data analysis using ^{1}H-NMR and ^{13}C-NMR

Atomic Number	Chemical Shift ^{1}H-NMR (ppm)	Integration and Type Multiplicity	Chemical Shift ^{13}C-NMR (ppm)
1	-	-	128.8
2/6	7.64 (J = 9.2 Hz)	2H, d	131.9
3/5	6.95 (J = 8.4 Hz)	2H, d	114.7
4	-	-	116.9
1’	-	-	120.8
2’	-	-	162.4
3’	6,97 (J = 8.8 Hz)	1H, d	120.3
4’	7.42 (J = 2.6 Hz; 8.8 Hz)	1H, dd	146.5
5’	-	-	127.1
6’	7.85 (J =2.4 Hz)	1H, d	136.0
Cα	7.44 (J= 15.2 Hz)	1H, d	123.5
Cβ	7.92 (J =16 Hz)	1H, d	162.4
C=O	-	-	192.8
OCH_3	3.87	3H, s	55.5
OH	12.85	1H, s	-

The ^{1}H-NMR spectra at a shift of 7.92 ppm had a coupling constant of 16 Hz. At a shift of 7.44 ppm, the coupling constant is 15.2 Hz. This proves that chalcone compounds have been formed due to unsaturated α, β bonds that have been confirmed by ^{1}H-NMR data. Then at a shift of 3.87 ppm, there is a singlepeak which has 3 H atoms when viewed from the integral results. It is estimated that this peak comes from the H atom attached to the methoxy group in the para position. It is known that the H atom attached to the methoxy group is in a shift of 3.7-4.3 ppm. At a shift of 12.85 ppm there is a single peak with the result of the integration of 1 proton which is thought to be a proton in the hydroxyl group attached to the B-chalcone ring.

The analysis using ^{13}C-NMR showed that there were 16 carbon atoms. The 55.5 ppm shift shows the methoxy carbon atom bonded to the aromatic A ring. It is known that the C sp3 proton shift is 10-30 ppm, the shift is bigger because the C atom is attached to the O atom, so it is less protected by electrons and causes the proton to shift more. At a shift of 114.3-162.4 ppm there are 10 signals which are carbon signals from ring A, ring B and two carbons that form α, β unsaturated bonds because according to the existing theory C sp^2 atoms have a shift of 110-175 ppm. At a shift of 192.7 ppm there is a signal that is thought to be C carbonyl. The characteristic of C carbonyl in the ketone group is in the range of 190-220 ppm. Based on the results of the analysis of IR spectroscopy, mass spectroscopy, and Nuclear Magnetic Resonance (NMR) of chlorocalcone compounds with the trivial name 2'-hydroxy-5'-chloro-4-methoxychalcone were successfully synthesized.

Toxicity Test

The results of the toxicity test for 2'-hydroxy-5'-chloro-4-methoxychalcone were obtained using the Brine Shrimp Lethality Test (BSLT) method against *Artemia salina* Leach and analyzed using the Reed and Muench method. Based on the test results, the compound 2'-hydroxy-5'-chloro-4-methoxychalcone has an LC_{50} value of 75.07 ppm and has the potential as an anticancer, antibacterial, antifungal, antioxidant and so on.

Antioxidant Activity Test

Antioxidant testing was also carried out using the DPPH method, the compound 2'-hydroxy-5'-chloro-4-methoxychalcone has the potential as a very strong antioxidant with an IC_{50} value of 45.99 ppm. This compound has a methoxy, hydroxyl group, Cl substituent and contains a reactive keto ethylene group (-CO-CH=CH-). The presence of these groups causes chalcone compounds to have various kinds of biological activities.

CONCLUSION

It has been successfully synthesized a chlorocalcone compound, namely 2'-hydroxy-5'-chloro-4-methoxychalcone with a yield of 84.54%. The results of bioactivity and antioxidant tests showed that the synthesized compound had an LC_{50} value of 75.07 ppm and an IC_{50} of 45.99 ppm.

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