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PHARMACEUTICAL POTENTIAL OF 2',4'-DICHLORO-4-HYDROXY-3-METHOXYCHALCONE SYNTHESIZED FROM VANILINE

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ABSTRACT

Chalcone is an intermediate compound as the main precursor for the biosynthesis of flavonoid in plants. Chalcone has been known to have variety of different pharmacological activities. The difference in chalcone activity is influenced by the differences of subtituents found in both aromatic rings on the chalcone stucture. This study performed the synthesis of 2',4'-dichloro-4-hydroxy-3-methoxychalcone compound from the raw material of 2,4-dichloro acetophenone and vanillin by Claisen-Schmidt reaction using conventional method by stirring. The yield of synthesized compound is 91.57% of purity. The synthesized compounds were characterized by structural elucidation methods using IR, MS, ^{1}H-NMR and ^{13}C-NMR. Toxicity and antioxidant activity tests were performed on the synthesized compound. Base on the test results obtained LC_{50} value of 20.04 ppm and IC_{50} 26.10 ppm. It is better to describe the pharmaceutical potential of 2',4'-dichloro-4-hydroxy-3-methoxychalcone little bit further.

Keywords: 2,4-dichloro acetophenon, 2',4'-dichloro-4-hydroxy-3-methoxychalcone, chalcone, synthesis, vanillin

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INTRODUCTION

Chalcone or 1,3-diphenyl-2-propena-1-one, is a compound contain two aromatic rings connected by three carbon atoms in α,β-unsaturated carbonyl system [1]. Chalcone is an important group of natural products, and some of them have various biological activities, such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-tumor, antioxidant, and act as tyrosinase inhibitors [2]. The double bond between two aryl groups on chalcone structure plays an important role in the activity of the chalcone. It's difficult to isolate chalcone from plants because of the chalcone sintetase enzyme (CSH) which immediately converts the chalcone into flavonoids [3].

The synthesis of chalcone has been reported from the reaction of acetophenone with aromatic aldehyde in the presence of potassium hydroxide catalysts and ethanol

at room temperature by the Claisen-Schmidt condensation method [4]. The synthesis of chalcone can be one of the most promising alternative method of obtaining chalcone when the isolation of chalcone from the natural material is difficult or the yield is too small.

The study performed by synthesizing chalcone from vanilin and 2,4-dichloroacetophenone as base material by Claisen-Schmidt reaction with NaOH catalyst at room temperature. Antioxidant and toxicity test were performed on the resulting chalcone compound.

METHODS

Tool

Analytical scales, a set of glass tools, magnetic stirrer, buchner funnel, vortex, vacuum pump, micropipette, aquarium, UV-Vis spectrophotometer Dynamica Halo DB-20S, JMM JEM JNMECA 500 MHz, MS QP2010S, Shimadu IR ShimadzunPrestige-21.

Material

Vanillin, DPPH, 2,4-dichloro acetophenone, Artemia Salina L eggs, tween 80, NaOH 40%, 10%HCl, ethanol, methanol, aquades, sea water and whattman paper.

Synthesis of Chalcone Compound

0.945 grams (5 mmol) of 2,4-dichloro acetophenone and 0.760 (5 mmol) grams of vanillin were each dissolved in 10 mL of ethanol. Both solutions were incorporated into a closed erlenmeyer flask and added 5 mL of NaOH. The mixture was stirred at a constant velocity. The reaction was controlled using thin layer chromatography (TLC) every 3 hours. The reaction stopped after 24 hours. 10% HCl added to the mixture until the pH of the mixture reaches 7. Whattman paper was weighed using an analytical scale. The mixture was filtered using a buzzer funnel coated with whattman paper.

Structure Characterization

The structure of the synthesis compounds was characterized by structural elucidation methods using infrared spectroscopy, mass spectroscopy and nuclear magnetic resonance (NMR).

Brine Shrimp Lethality Test (BSLT)

0.3 grams of *Artemia salina* Leach eggs inserted into the aquarium containing 500 mL of sea water. The aquarium was placed under a 5 watt lamp and is equipped with an aerator. After 48 hours the eggs hatch into a ready-to-use larva for BSLT.

The solution of the chalcone in seawater was made with a concentration of 50 ppm as the parent solution. 75 vials were each filled with 10 larvae of Artemia salina L. Subsequently, a number of parent solutions were added suitable for making series concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm into each vial with 5 replications. Sea water was added into the vial to complete the volume of the solution to 10 mL. After 24 hours, observation has done to count the number of dead larvae in each vial. The data obtained were analyzed using Reed and Muench analysis method to determine the LC_{50} value.

Antioxidant Activity Test

The solution of the chalcone in methanol was made with a concentration of 50 ppm. Soncentration series of chalcone solution with concentration of 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm made from parent solution. A total of 2 ml of each concentration series solution were inserted into each reaction tube with 5 replications.

DPPH solution in methanol was made with a concentration of 40 ppm. A total of 2 mL DPPH was inserted into a reaction tube previously filled with a solution of chalcone. Incubation of DPPH

and chalcone mixture was carried out for 30 minutes.

DPPH absorption was measured using a UV-Vis spectrophotometer at 515 nm wavelength. The absorbance data obtained were analyzed using linear regression analysis to determine IC_{50} values.

RESULT AND DISCUSSION

Synthesis of Chalcone Compound

The compound obtained from the synthesis has a physical characteristic of a yellow solid. The weight of the resultant synthesis compound was 1.48 g. The percentage of the synthesis yield is 91.57% based on the calculation using percent formula of yield. The greater percentage of the rendement signifies the greater the likelihood of obtaining the compound in large quantities. The yield of this synthesis can be used as a basis for consideration to produce a suitable 2',4'-dichloro-3-hydroxy-4-methoxychalcone compound in accordance with the requirement.

The reaction of the formation of the 2',4'-dichloro-3-hydroxy-4-methoxy chalcone compound begins with the formation of a nucleophile carbanion of the 2,4-dichloroacetophenone. The hydroxy ion in the NaOH catalyst will attract 1 hydrogen atom from Cα in the 2',4'-dichloro acetophenone compound to form a nucleophile carbanion. After the nucleophile properties of the 2',4'-dichloroacetophenone were formed, the reaction continued with the addition of the vanillin compound. The vanillin compound contains a C-carbonyl atom in the form of an aldehyde group having a low electron density due to the influence of the oxygen atom. Consequently a nucleophile carbanion will attack C-carbonyl in the structure of the vanillin compound which causes the incorporation of the 2'4-dichloro acetophenone and vanillin compounds into a 2',4'-dichloro-3-hydroxy-4-methoxy chalcone compound. The merging of both releases 1 water molecule to achieve the stability of the compound structure.

Structure Characterization

Structure characterization of the synthesized compund was done by structural elucidation method using infrared spectroscopy, mass spectroscopy and nuclear magnetic resonance (NMR).

Mass spectroscopy was performed to determine the relative molecular mass of the compound obtained from the synthesis. Infrared spectroscopy was performed to determine the functional groups present in the synthesized compound. Nuclear magnetic resonance (NMR) was performed to determine the number of hydrogen (H) and carbon (C) atoms and their environment in the structure of the compound.

The results of mass spectroscopy showed that the relative molecular mass of the compound of the synthesized product is 322 g/mol. Theoretically, the molecular mass of 2',4'-dichloro-3-hydroxy-4-methoxychalcone compound can be calculated by adding the relative molecular mass of the 2,4-dichloro acetophenone and vanillin compound molecules subsequently subtracted by the relative molecular mass of water. Based on theoretical calculations the relative molecular mass of the 2',4'-dichloro-3-hydroxy-4-methoxychalcone compound is 322 g/mol. Thus, based on the results of mass spectroscopy analysis, the relative molecular mass of the synthesized compound has corresponded to the relative molecular mass of 2',4'-dichloro-3-hydroxy-4-methoxychalcone compound.

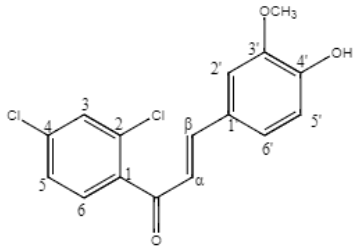

Figure 1. Structural compound of 2',4'-dichloro-3-hydroxy-4-methoxychalcone

Table 1. Infrared spectra of 2',4'-dichloro-3-hydroxy-4-methoxychalcone

V (cm^{-1})	Functional groups
740,7	C-Cl
979,8	C=C trans
1165	OCH_3
1280,7	C-O fenol
1581,6	C=C aromatik
1643,4	C=O
3402,4	OH

Table 2. ^{1}H-NMR and ^{13}C-NMR spectra of 2',4'-dichloro-3-hydroxy-4-methoxychalcone

Atom Number	δ ^{1}H-NMR (ppm)	Integration and Multiciplity type	δ ^{13}C-NMR (ppm)
1	-	-	132,4
2	-	-	136,8
3	7,45 (*J* = 2Hz)	1H, d	130,3
4	-	-	137,8
5	7,32 (*J* = 8,35; 2 Hz)	1H, dd	126,9
6	7,38 (*J* = 8,35 Hz)	1H, d	130,4
1'	-	-	127,4
2'	7,03 (*J* = 2 Hz)	1H, d	109,0
3'	-	-	149,0
4'	-	-	147,1
5'	6,90 (*J* = 8 Hz)	1H, d	115,1
6'	7,08 (*J* = 8 ; 2 Hz)	1H, dd	123,9
A	6,92 (*J* = 16 Hz)	1H, d	124,2
B	7,33 (*J* = 16 Hz)	1H, d	147,5
C=O	-	-	193,1
OCH_3	3,90	3H, s	56,2
OH	6,07	1H, s	-

The results of infrared spectroscopy (Table 1) indicate the suitability of the functional groups present in the synthesized compound with the compound of 2',4'-dichloro-3-hydroxy-4-methoxychalcone. There is a peak at wave number 740.7 cm^{-1} which is the region of the wave number of carbon atoms attached

to the chlorine atoms. The wave number 979.8 cm^{-1} indicates the wave number of the C = C group with the trans position. This data is important because it shows the formation of a typical C = C transitional position of a chalcone compound which is not present in the two basic materials. Peak at wave number 1165 cm^{-1} indicates the presence of methoxy groups in A ring of the compound of 2 ', 4'-dichloro-3-hydroxy-4-methoxichalcone. The existence of peak at wave number 1280.7 cm^{-1} which is area of waves of C-O group of phenol compound. Peak at wave number 1581.6 indicates the wavelength of the C = C group on the aromatic ring. Peak at wave number 1643.3 cm^{-1} indicates the presence of C=O groups in the compound being analyzed. There was a shift of wave numbers in the C=O group of compounds analyzed. This data shows the wave number of the C=O group in the ketone enon structure of the chalcone compound instead of C=O on the base material. Because C=O is generally at a wave number around 1700 cm^{-1}. The peak at wave number 3402.4 cm^{-1} indicates the presence of OH group on the compound being analyzed. All functional groups identified in infrared spectra indicate conformity to the functional groups of the designed 2',4'-dichloro-3-hydroxy-4-methoxychalcone compound.

^{13}C-NMR data (Table 2) shown that the synthesized compound had carbon atoms (C) amounted to 16 with the specification C-sp^3 as much as 1, C-sp^2 (C = O) as much as 1 and C-sp^2 (C = C) as much as 14. ^{1}H-NMR data indicates that the compound has 12 hydrogen atoms (H) and the resulting spectra correspond to the hydrogen atom environment of the 2',4'-dichloro-3-hydroxy-4-methoxychalcone compound. Data on chemical shifts of 6.92 ppm (J = 16 Hz) and 7.33 ppm (J = 16 Hz) indicated the formation of typical C = C bonds of chalcone with trans configuration because it has the same coupling constant of 16 Hz. The same coupling constant indicate that both protons affect each other and are in the same adjacent position or environment. Generally protons with trans configuration will produce 12-19Hz coupling constants. The data on the chemical shifts of 7.45 ppm (J = 2 Hz), 7.32 ppm (J = 8.35, 2 Hz), 7.38 ppm (J = 8.35 Hz) with the integration of each 1 have corresponding coupling constants. It also explains that the three protons are in the same environment that is in the ring A in the 2',4'-dichloro-3-hydroxy-4-methoxychalcone. The relationship of 3 protons to the ring B shows the chemical shift data of 7.03 ppm (J = 2 Hz), 6.90 ppm (J = 8 Hz), 7.08 ppm (J = 8; 2 Hz) which also has corresponding coupling constant. Data on a chemical shift of 3.90 ppm with singlet and singular integrity 3 indicate the presence of the OCH_3 group. Data on a chemical shift of 6.07 ppm with singlet multiplicity and integration 1 indicates the presence of OH groups.

Based on the results of analysis known 2',4'-dichloro-3-hydroxy-4-methoxychalcone has been formed through the reaction between vanillin and 2,4-dichloro acetophenone in alkaline atmosphere.

Brine Shrimp Lethality Test (BSLT)

The Brine Shrimp Lethality Test (BSLT) is an early screening by looking at the toxicity of the compound against the *Artemia salina* L larvae to know whether or not biological activity of a compound by determine LC_{50} values. LC_{50} is the concentration of the compound which can kill 50% of the population in this case the shrimp larvae *Artemia salina* L. The LC_{50} value is determined from the data of dead larvae and living animals, each of which is accumulated (Table 3). The data were then analyzed using Reed and Muench analysis method. The result of analysis shows that

the LC_{50} value of synthesized compund is 20.04 ppm.

Based on the value of LC_{50} obtained from the analysis, synthesized chalcone has the potential to move as a cytotoxic agent (anticancer). Compounds with values of $LC_{50} \leq 30$ ppm are active compounds that have potential as anticancer [5]. The 2',4'-dichloro-4-hydroxy-3-methoxychalcone is an active biological compound and has potential as an anticancer. This is because the 2',4'-dichloro-4-hydroxy-3-methoxychalcone compound has a keto ethylene group which makes the chalcone compound into a biologically active compound. The activity of chalcone derived compounds can be increased by increasing the stability of ethylene keto groups. This may be due to chloro subcituents at positions 2 and 4 on ring B, methoxy at position 3 on ring A, and hydroxy at position 4 on ring A, may increase the stability of ethylene keto groups in the 2',4'-dichloro-4 -hydroxy-3-methoxychalcone. Thus, the 2',4'-dichloro-4-hydroxy-3-methoxychalcone compound becomes a compound having good bioactivity.

Table 3. Reed and Muench analysis 2',4'-dichloro-3-hydroxy-4-methoxychalcone

Concentration (ppm)	Number of		Total		Ratio x/(x+y)	Mortality (%)
	Alive	Death	Alive	Death		
	47	3	173	3	0.017	1.70
10	42	8	126	11	0.080	8.02
15	35	15	84	26	0.236	23.66
20	28	22	49	48	0.494	49.48
25	21	29	21	77	0.785	78.57

Tabel 4. Antioxidant activity of 2 ', 4'-dichloro-4-hydroxy-3-methoxychalcone

Concentration (ppm)	Activity (%)	IC_{50}
10	27.03%	
20	54.07%	
30	69.62%	26.10 ppm
40	79.81%	
50	84.81%	

Antioxidant Activity

The results showed IC_{50} value (Table 4) of 2',4'-dichloro-4-hydroxy-3-methoxy chalcone is 26.10 ppm. Based on these results 2',4'-dichloro-4-hydroxy-3-methoxychalcone classified as a compound that has very strong antioxidant activity. Compounds with $IC_{50} \leq 50$ ppm are compounds that have high capacity as antioxidants. Thus the 2 ', 4'-dichloro-4-hydroxy-3-methoxychalcone compound is a compound that has a high capacity as an antioxidant.

The antioxidant activity of the synthesized chalcone compound is probably due to the presence of a hydroxy subtituent at position 4 in A ring of 2 ', 4'-dichloro-4-hydroxy-3-methoxychalcone.
A hydroxy group bound to an aromatic ring has the ability to donate an electron by

releasing a hydrogen atom. So that one electron released in the form of a hydrogen atom from the structure of the compound can satisfy the electrons present in the radical compound. In addition the antioxidant activity of the 2',4'-dichloro-4-hydroxy-3-methoxychacone compound is also supported by the presence of a conjugated double bond on the structure of the compound.

CONCLUSIONS

The chalcone derivate compound has successfully synthesized from vanilin base material and 2,4-dichloro acetophenone through Claisen-Schmidt reaction using NaOH catalyst at room temperature with yield 91.57%. The 2',4'-dichloro-4-hydroxy-3-methoxichalcone has the potential as an anticancer agent with LC_{50} value of 20.04 ppm and as an antioxidant agent with IC_{50} value of 26.10 ppm.

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