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Synthesis of 7-(Hydroxy) Coumarin and Its Activity Test As Antibacterial against *Staphylococcus aureus* and *Shigella flexneri*

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Abstract. *Prabawati S Y, Fitriana. 2017. Synthesis of 7-(Hydroxy) Coumarin and Its Activity Test as Antibacterial against Staphylococcus aureus and Shigella flexneri. Proc Internat Conf Sci Engin 1: 141-145.* A synthesis of 7-(hydroxy)coumarin and its activity as antibacterial against *Staphylococcus aureus* and *Shigella flexneri* has been performed. The synthesis of the coumarin was carried out by reacting ethyl acetate and 2,4-dihydroxybenzaldehyde using piperidine as an alkaline catalyst via Knoevenagel reaction and antibacterial activity test was performed using disc diffusion method. The product of 7- (hydroxy)coumarin was obtained as a bright brown crystalline (m.p. 125 °C) with a rendment of 77%. The FTIR spectrum shows the absorption of -OH groups at 3101,54 cm^{-1} and unsaturated lactone groups at 2345.44 cm^{-1} which is a typical group of coumarin's derivatives. Analysis using ^{1}H-NMR spectrometer also showed the proton of the -OH group appearing on chemical shift (δ) 9.932 ppm. The antibacterial activity test showed that the compound of 7- (hydroxy) coumarin has the highest activity at 20% concentration as an antibacterial against bacteria *Staphylococcus aureus* and *Shigella flexneri* with inhibit zone was 24.55 and 20.43 mm, respectively. It means the 7-(hydroxy) coumarin compound has a strong inhibiting activity.

Keywords: 7- (hydroxy)coumarin, antibacterial, *Staphylococcus aureus*, *Shigella flexneri*

INTRODUCTION

The coumarin compounds are commonly found in nature including in some plants, such as in strawberries, cherry, sinamon, and lavender (Aslam et al, 2010). The coumarin derivatives are known to have biological activity such as blood anticoagulant, antibiotic, anticarcinogenic (Copriyadi, 2005), antioxidant, antimicrobial, antifungal (Abdou, 2014), and as antibacterial (Sashidhara, 2010). Sahoo *et al* (2014) has reported that the 4-hydroxy-3- (pyridin-2-yldiazenyl) coumarin compound can be used as an antibacterial to *Staphylococcus aureus* with a very strong inhibiting area.

Coumarin is a lactone compound of orthokumaric phenolic compound (an ortho hydroxy cinnamate), and when the phenolic group is bound to a glucose molecule a glycoside molecule is formed which is a bonded cumarin 6. A simple coumarin is a phenylpropanoid containing a benzene ring C-6 with an aliphatic C-3 as a side chain. (Alegantina and Ani, 2010).

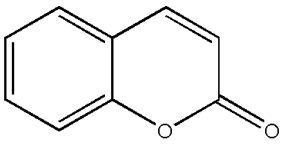

Figure 1. Structure of the Coumarin Compound.

Coumarin synthesis and its derivatives can be performed by using Knoevenagel reaction. The 3-acetyl-6-Bromo-2H-chromen-2-one was synthesized by Knoevenagel reaction by reacting 5-bromo salicylaldehyde and ethyl acetoacetate using piperidine as a catalyst. The synthesis results obtained randemen of 96% and has activity as an antioxidant. Other coumarin derivatives such as 3-(2-bromoacetyl)-2H-chromen-2-one are obtained from the reaction between 3-acetyl-6-Bromo-2H-chromen-2-one and hydrogen bromide. This compound apparently has activity as an antibacterial (Kasumbwe, 2014).

In this experiment, synthesis of 7 (hydroxy)coumarin was obtained through Knoevenagel reaction with ethyl acetate and 2,4-dihydroxybenzaldehyde as starting materials and piperidine as a catalyst. According to Aslam *et al* 2010, coumarin derivative compounds are more active inhibiting *Staphylococcus aureus* bacteria compared with *Escherichia coli*. So in this study, *Staphylococcus aureus* used as Gram positive bacteria and *Shigella flexneri* as Gram negative bacteria.

Changes in the basic structure of the coumarin may have an effect on their biological activity. The synthesis of cumarin derivatives by adding pharmacophoric groups at C-3, C-4 and C-7 positions enables them to be used as antimicrobial, anti-HIV, anticancer, antioxidant and anticoagulant (Dighe *et al*. 2010). Therefore, in this study the addition of hydroxyl groups at C-7 allows the 7-(hydroxy)coumarin compounds to be used to inhibit the growth of *Staphylococcus aureus* and *Shigella flexneri* bacteria.

MATERIALS AND METHODS

Experimental Equipments

The Experimental equipments used in this research were laboratory glassware, a set of recrystallization

equipment, analytical balance (OHAUS), hot plate (CIMAREX), oven, petri dish, microwave, autoclave, melting point, infrared spectrophotometer (Shimadzu FTIR-8201 PC) and ^{1}H-NMR spectrometry (Agilent Technologies 500 MHz).

Experimental Materials

The materials used in this study were piperidine, 2,4-dihydroxybenzaldehyde, ethanol, ethyl acetate, methanol, HCl, aquadest, *Staphylococcus aureus* bacteria, *Shigella flexneri* bacteria, Nutrient Agar (NA) and Nutrient Broth (NB). All materials with analytical grade quality except aquadest.

Experimental Procedure

Synthesis of 7-(Hydroxy)Coumarin

A total of 0.047 mol (4.14 mL) of ethyl acetate and 0.005 mol (0.69 grams) of 2,4-dihydroxy benzaldehyde compound were introduced into the beaker with a mole ratio of 6: 1. The mixture was continuously stirred and piperidine was added and stirred again for 1-2 hours at room temperature until yellow solution was formed. Then added 3 mL of HCl and 5 mL of ethanol. The mixture was stirred again and heated. After that stand for 24 hours until formed yellow crystals. The resulting crystals were filtered and subsequently recrystallized with ethanol and methanol. The purity test was performed with melting point determination and product was characterizating using FTIR spectrophotometer, and ^{1}H-NMR spectrometer.

Test Activity as Antibacterial Substance

A total of 4.0 grams of NA media and 1.6 grams of NB media were included in the erlenmeyer and added 1000 mL of distilled water and then heated with microwave to boiling. Furthermore erlenmeyer mouth closed with cotton and with paper tied with rubber band, then sterilized by autoclave at temperature 121 ^{o}C for 15 minutes.

Reculture of bacteria was carried out in the laminar air flow that has been sterilized. *Staphylococcus aureus* and *Shigella flexneri* bacteria were each we of antibacterial activity of *Staphylococcus aureus* and *Shigella flexneri* was performed using diffusion method with paper disc. Bacterial inoculation was done by pour plate method. Each of the prepared paper disks was immersed in the synthesis compound with concentrations of 5, 10, 20 and 40% (w/v), penicillin as a positive control and DMSO as a negative control. The discs have been added to various concentrations, then placed on the media by pressing the disc paper. The cup was then incubated for 24 hours at 37 °C. After incubation, the barrier zone was observed and measured by the sliding range. The lowest concentration that can inhibit bacterial growth is expressed as the value of Minimum Inhibitory Concentration (MIC). (Capuccino and Suherman, 2011).

RESULTS AND DISCUSSION

The synthesis of 7-(hydroxy)coumarin was carried out by reacting ethyl acetate and 2,4-dihydroxybenzaldehyde by a ratio of 6: 1 and added a few drops of piperidine as a catalyst. The reaction was carried out at room temperature by continuous stirring for ± 2 hours. Piperidine as an alkaline catalyst will attack Hα from ethyl acetate to form an enolate ion which will become a nucleophile.

The next reaction is an aldol condensation reaction between the enolate ions and 2,4-dihydroxybenzaldehyde as shown in Figure 1 (a). The product of aldol condensation reaction undergoes dehydration reaction as shown in Figure 2.

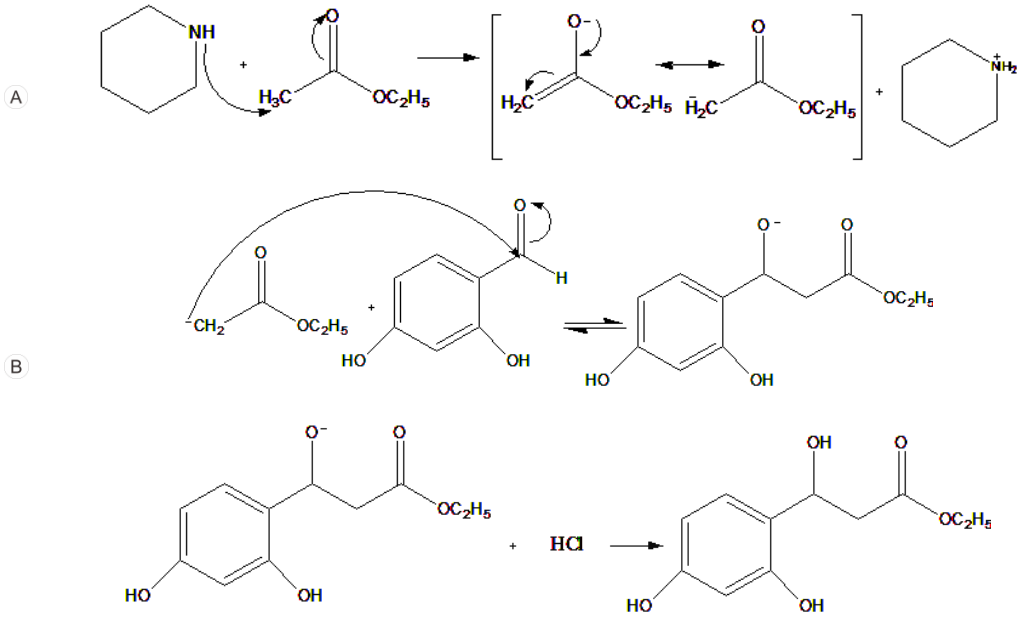

Figure 2. **A**. The mechanism of formation of an enolate, **B**. aldol condensation between enolate ions with 2,4-dihydroxybenzaldehyde.

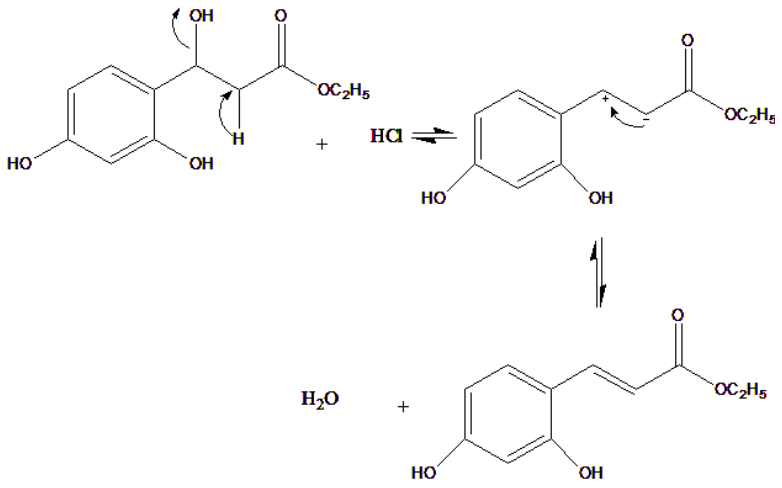

Figure 3. Mechanism of dehydration reaction.

The formation of 7-(hydroxy)coumarin was occured through a transesterification reaction mechanism i.e with the release of ester groups due to the addition of ethanol. The reaction mechanism is shown in Figure 4.

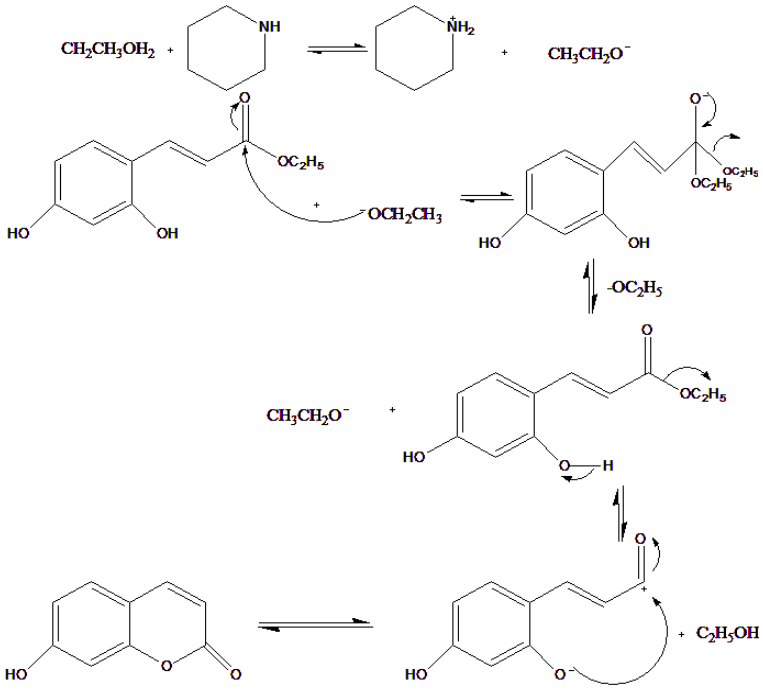

Figure 4. Mechanism of formation of 7-(Hydroxy)coumarin compound.

The synthesis product was then recrystallized to obtain a dark brown solid with a melting point of 125 ^oC. The identification of the product with FTIR spectrophotometer gives the spectrum as shown in Figure 5. From the spectrum, it is seen that there is a shift of absorption of -OH group from wave number 3132,40 cm^{-1} shifted to 3101,54 cm^{-1}. Meanwhile, the absorption of unsaturated lactone groups appears at wave numbers 2345,44 cm^{-1}. The absorption of the C(O)-C group, which is the characteristic group of the coumarin compound (Adfa, 2006) appears at wave numbers 1234.44 cm^{-1}.

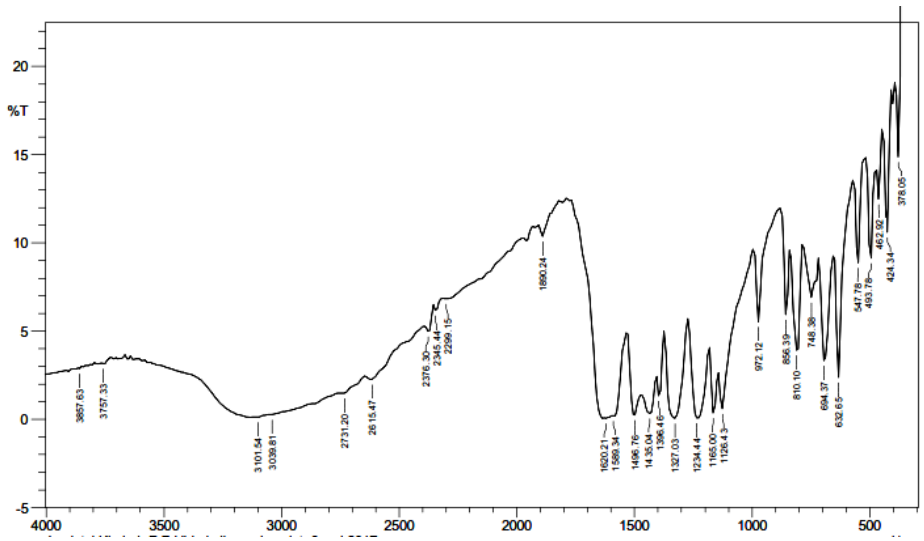

Figure 5. FTIR spectrum of 7-(hydroxy)coumarin and 2,4-dihydroxy-benzaldehyde.

Based on the ^{1}H NMR spectrum, there is an absorption at chemical shifts 6.34-6.39 ppm and 6.40-6.4 ppm which is the peak of H-3, H-6 and H-8 protons. The structure of 7- (hydroxy)coumarin is shown in Fig. 6. According to Mudjirahmini and Taslim (2007) in Guilet (2001), at the chemical shift of 5.92 ppm and 6.69 ppm is the peak of proton of aromatic compounds of coumarin at position of H-3 and H-6.

The peak in a chemical shift of about 7,50-7,52 ppm shows the protons of H-4 and H-5. This is in line with Ibrahim et al (2007) study on isolation of coumarin from pinang seed (Areca catechu L.), that H-5 uptake appears at a chemical shift 7,51 ppm. Meanwhile, the peak in the chemical shift 9.932 ppm with the singlet appearance is the proton absorption of the -OH group. According to Ibrahim et al (2007), proton absorption of –OH group is appear at a chemical shift 10.45 ppm with singlet appearance. Thus, it can be concluted that the compound of synthesis result is 7- (hydroxy)coumarin.

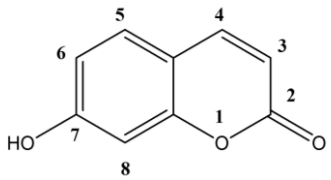

Figure 6. Structure of 7-(hydroxy)coumarin Compound.

The results of the antibacterial activity of 7-(hydroxy)coumarin against *Staphylococcus aureus* and *Shigella flexneri* bacteria as shown in Table 1.

Table 1. Results of the Antibacterial Activity Test of 7-(hydroxy)Coumarin on *Staphylococcus aureus* (Gram +) and *Shigella flexneri* (Gram -).

No	Concentration (% w/v)	inhibitor diameter (mm)	
		Gram +	Gram -
1	Negative control (DMSO)	-	-
2	Positive control (Penicilin 10)	29,82	30,00
3	5	13,54	13,57
4	10	20,10	19,78
5	15	21,86	19,99
6	20	24,55	20,43

Based on Table 1, it's known that the inhibitory zone diameter of the 7-(hydroxy)coumarin at a concentration of 20% has a very strong resistance area for both *Staphylococcus aureus* and *Shigella flexneri* bacteria. According to Davis and Stout (1971) in Ngajow *et al* (2013) it is known that when the diameter of the inhibit zone > 20 mm means having a strong activity, 16-20 mm inhibition diameter has medium activity, 10-15 mm inhibition diameter has weak activity, and the inhibitory diameter < 10 mm has very weak activity.

Based on data in Table 1, it is known that the 7-(hydroxy)coumarin has a smaller inhibitory ability against *Shigella flexneri* bacteria than *Staphylococcus aureus bacteria*. This is because the *Shigella flexneri* (Gram-negative) bacteria have an outer phospolipid membrane that maintains the structure of the lipopolysaccharide component so that the cell wall becomes impermeable to the antibacterial compound, whereas *Staphylococcus aureus* (Gram positive) has an uncomplicated cell wall, so that the 7-(hydroxy)coumarin is easier to damage the cell walls of *Staphylococcus aureus* bacteria.

Figure 7 shows the inhibitory zone of 7-(hydroxy)coumarin to *Staphylococcus aureus* and *Shigella flexneri* bacteria with MIC value of 5% (w/v).

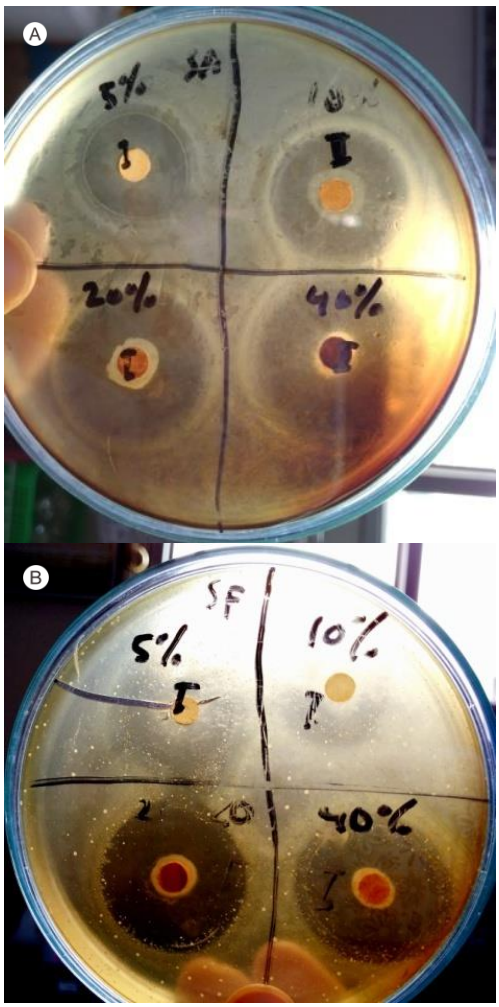

Figure 7. The Results of antibacterial test of 7-(hydroxy)coumarin against *Staphylococcus aureus* (**A**) and *Shigella flexneri* (**B**) bacteria at 24 hours and 37ºC.

The mechanism of inhibition of antibacterial compounds can be occured by triggering the inactivation of cellular enzymes that may alter membrane permeability. Excessive influx of extra cellular substances can trigger leakage of intracellular components including the release of K +, that is the first sign of damage. This may occur because the cumarin derived compound is a lactone compound of orthokumaric phenolic (an ortho hydroxy cinnamate) which can inhibit a bacterium by interfering with the activity of the enzyme.

CONCLUSIONS

The 7-(hydroxy)coumarin compound can be synthesized by Knoevenagel reaction using ethyl acetate and 2,4-dihydroxybenzaldehyde with piperidine catalysts. The product was obtained as a bright brown crystalline (m.p. 125 °C) with a rendment of 77%. The antibacterial activity test showed that the 7-(hydroxy)coumarin has potential as antibacterial to *Staphylococcus aureus* and *Shigella flexneri* with strong inhibit zone and 5% w/v MIC value.

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