# Synthesis and antibacterial activity test of 3-(3-(4-hydroxy-3-methylphenyl)akriloil) coumarin compounds

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# Synthesis and antibacterial activity test of 3-(3-(4-hydroxy-3-methylphenyl)akriloil) coumarin compounds

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**Abstract.** A 3-(3-(4-hydroxy-3-methylphenyl)acryloyl) coumarin has been synthesized from 4-hydroxy-3-methylbenzaldehyde with 3-acetylcoumarin. This research includes the synthesis of 4-hydroxy-3-metilbenzaldehid from *ortho*-cresol through Reimer-Tiemann reaction, synthesis of 3-acetylcoumarin from salicylaldehyde, synthesis of 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin, and antibacterial activity testing of 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin compound against *Escherichia coli* (gram negative bacteria) and *Staphylococcus aureus* (gram positive bacteria) using the disc diffusion method. The result of Reimer-Tiemann reaction was obtained as a yellow solid of 4-hydroxy-3-methylbenzaldehyde with yield of 31.91%. The synthesis of 3-acetylcoumarin was obtained as a yellow solid with a yield of 95.57%. A 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin was obtained as a brownish yellow solids with yield of 11.66%. A 3-(3-(4-hydroxy-3-methylphenyl)acryloyl) coumarin has better antibacterial activity than 3-acetylcoumarin compound against *Escherichia coli* and *Staphylococcus aureus.*
**Keywords:** Reimer-Tiemann Reaction, 3-acetylcoumarin derivatives, Antibacterial

## 1. Introduction

Coumarin is a heterocyclic compound that has a six-ring of lactone and contains a chromene core or 2H-1-benzopyran-2-on with the molecular formula $C_9H_5O_2$ [1]. Chromene is more commonly known as benzopyran. It is a polycyclic organic compound which is produced from the incorporation of benzene ring with pyran ring [2].

The coumarin derivatives compounds are obtained through an aldol condensation reaction. From the reaction, carbonyl group and aldehyde come from different compounds. Reactions are carried out with certain solvents in alkaline conditions [3]. Some researchers report that coumarin derivatives have antibacterial activity. Jayashree *et al.* [4] explained that coumarin derivatives have biological activities, such as anti-inflammatory, antioxidant, anti-allergic, antibiotic, antiviral, antibacterial, anticancer and show inhibitory effects of carcinogens. The types of substituents that are bound to the coumarin structure often affected to their activity as antibacterials. Lakshminarayanan *et al.* [5] and Helmy *et al.* [6]reported that one of the compound with the higher antibacterial activity was 3-aryl-acetylcoumarin derivative compound that was obtained from reaction between 3-acetylcoumarin with aldehyde aromatic compound. Some substituents were able to increase their activity as antibacterials. They were the substituents with electron lone pair such as hydroxy, methoxy and alkyl in the ring of coumarin compound. On the other hand, chalcone with substitution of OH and $CH_3$ groups have antibacterial

activity [7]. Rodriguez *et al.* [8] reported that 3-acetylcoumarin compound reacted with benzaldehyde derivatives will produce coumarinil chalcone compounds.

A 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin compound that was containing hydroxy and methyl substituents are obtained from the reaction between 4-hydroxy-3-methylbenzaldehyde with 3-acetylcoumarin. The presence of substituent of -OH and -$CH_3$ in the compound (3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin can increased to its antibacterial activity [9].

## 2. Methods

### *2.1. Materials and tools*

The ingredients in this research are *ortho*-cresol, salicylaldehyde, ethanol p.a, NaOH solids, distilled water, chloroform p.a, 1 M HCl solution, G60 silica gel, n-hexane p.a, ethyl acetate p.a, technical n-hexane, technical ethylacetate, anhydrous $Na_2SO_4$, ethylacetoacetate, piperidine, paper disc, yeast extract, peptone, nutrient agar, *S.aureus* and *E.coli* bacteria stock.

The laboratory equipment in this research are reflux devices, glassware, a set of column chromatography, Whatman filter paper, evaporator, *Ohaus* analytical balance, petri dishes, ose needle, tweezer, incubator, thermometer, autoclave, chamber, thin layer chromatography (TLC) plates, UV-Vis spectrophotometry, Fourier Transform Infrared (FTIR), Gas Chromatography Mass Spectroscopy (GC-MS), Liquid Chromatography Mass Spectroscopy (LC-MS) and H-NMR Spectrophotometry

### *2.2. Synthesis of 4-hydroxy-3-methylbenzaldehyde*

Synthesis of 4-hydroxy-3-methylbenzaldehyde was done by reacting (50 mmol, 5.15 mL) *ortho*-cresol in 100 mL ethanol with (75 mmol, 15 g) NaOH in 15 mL distilled water. Then, the mixture was heated at temperature 60°C. After 5 minutes' reaction, (100 mmol, 20 mL) chloroform was slowly added to the mixture dan stirred for 3 hours. Then, the reaction was monitored using thin layer chromatography plates (TLC plates) with the solvent system (ethyl acetate: n-hexane 1:3). After that, product from the reaction was evaporated immediately. Then, 1 M HCl solution was added to the product to give pH range in about 2-3 and extracted with ethyl acetate. The ethyl acetate extract was dried with anhydrous $Na_2SO_4$ and evaporated. Furthermore, the product was purified using column chromatography [10, 11]. The last stage, product was identified using by GC-MS, FT-IR, and H-NMR.

### *2.3. Synthesis of 3-acetylcoumarin*

Synthesis of 3-acetylcoumarin was done by reacting (0.08 mol, 8.73 mL) salicylaldehyde, (0.09 mol, 12.52 mL) ethylacetoacetate, and (0.5 mL) piperidin and stirred at room temperature for 5 minutes. Then, the reaction was monitored using thin layer chromatography plates (TLC plates) with the solvent system (ethyl acetate: n-hexane 1:3). Then, the mixture was extracted with ethyl acetate. The ethyl acetate fraction was added with anhydrous $Na_2SO_4$ then evaporated until the solvents run out [3, 4]. The last stage, product was identified using by UV-Vis spectrophotometry and H-NMR.

### *2.4. Synthesis of 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin*

The mixture of (0.007 mol, 1.4 g) 3-acetylcoumarin, (0.007 mol, 1.0 g) 4-hydroxy-3-methylbenzaldehyde, and 7.4 mL ethanol in alkaline conditions by adding 0.5 mL piperidine and heated at temperature 60°C for 3 hours. Then, the reaction was monitored using thin layer chromatography plates (TLC plates) with the solvent system (ethyl acetate: n-hexane 1:3). Product from the reaction was added by some drops of 1M HCl solution until pH range 2-3 and extracted with ethylacetate. After that, ethyl acetate fraction was added by some of anhydrous $Na_2SO_4$. Then, product was filtered and evaporated until the solvents run out [3, 6]. Furthermore, the product was purified using column chromatography and identified by using UV-Vis spectrophotometry, FT-IR and LC-MS.

### *2.5. Antibacterial activity test*

Antibacterial activity test of 3-3-(4-hydroxy-3-methylphenyl) acryloyl)coumarin compound was done by using diffusion disc method based on measuring of minimum inhibitory concentration [12]. The compound that being tested in this research are 3-3-(4-hydroxy-3-methylphenyl)acryloyl) coumarin, 3-acetylcoumarine, amoxicillin (positive control) and DMSO (negative control). Each compound was dissolved in DMSO with a concentration 2000 ppm. Then, they were tested as antibacterials against gram-positive bacteria (*S.aureus*) and gram-negative bacteria (*E.coli*).

## 3. Results and Discussion

### *3.1. Synthesis of 4-hydroxy-3-methylbenzaldehyde*

The synthesis of 4-hydroxy-3-methylbenzaldehyde were obtained from phenol derivative namely *ortho*-cresol. This compound was produced through the Reimer-Tiemann reaction using chloroform and NaOH as reagent. Reimer-Tiemann reaction is an aldehyde group formation on the aromatic nucleus along with attacking of the electrophilic dichlorocarbene on the aromatic nucleus. Then, it was followed by the hidrolisis reaction of dichloromethyl that was formed into *ortho* and *para* hydroxy benzaldehyde [13]. The reaction of the formation of 4-hydroxy-3-methylbenzaldehyde compound is presented in Fig. 1.

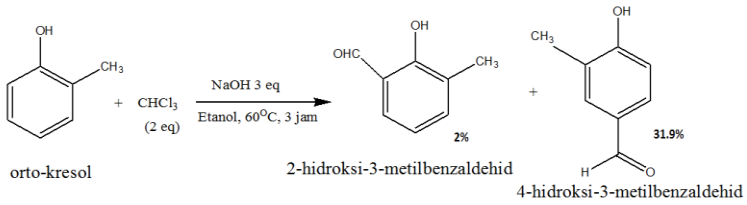


**Figure 1**. Reaction of the formation of 4-hydroxy-3-methylbenzaldehyde.

Product 4-hydroxy-3-methylbenzaldehyde were analysed using GC-MS to determine the total of compounds quantitatively, structural fragmentations, and molecular weight of compounds with the different of retention times.

The GC-MS analysis result is presented in Fig. 2. The highest percentage of abundance is *para* product (4-hydroxy-3methyl-benzaldehyde) in about 53% with $t_R$ (retention time) about 25.068 minutes. While *ortho* product (2-hydroxy-3-methylbenzaldehyde) in about 2% with $t_R$ (retention time) about 14.213 minutes. On the other hand, *ortho*-cresol as starting material still remains about 27%. While the other abundance about 18% is 4-dichloromethyl-4-methyl-2,5-cyclohexadiene compound as the result of side reactions from synthesis.

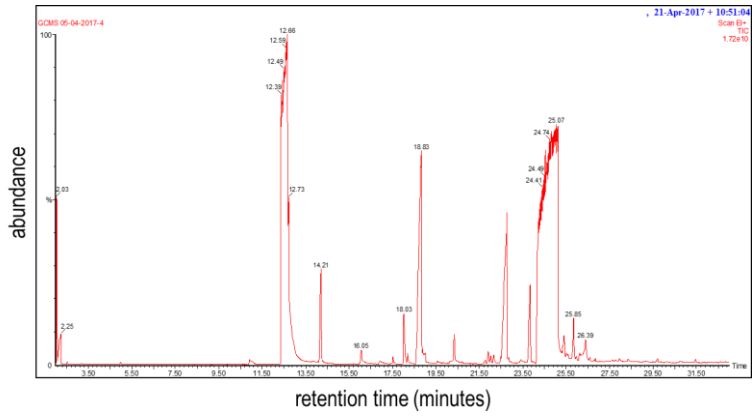


**Figure 2.** GC-MS chromatogram of 4-hydroxy-3-methylbenzaldehyde

Then, products form the synthesis were purified by column chromatography with ethyl acetate: n-hexane as solvent, then each compound was identified using TLC to compare the Rf value of the compound with *ortho*-cresol and salicylaldehyde. The first fraction was 2-hydroxy-3-methylbenzaldehyde, the second fraction was *ortho*-cresol compound as residual from starting material, the fourth fraction was 4-hydroxy-3-methylbenzaldehyde because it has a Rf value which was far from the standard. Both of the aldehyde compounds were identified using H-NMR to determine the type of proton in each compound. The H-NMR spectra of 2-hydroxy-3-methylbenzaldehyde compound is presented in Fig. 3. While the H-NMR spectra of 4-hydroxy-3-methyl benzaldehyde compound is presented in Fig. 4.

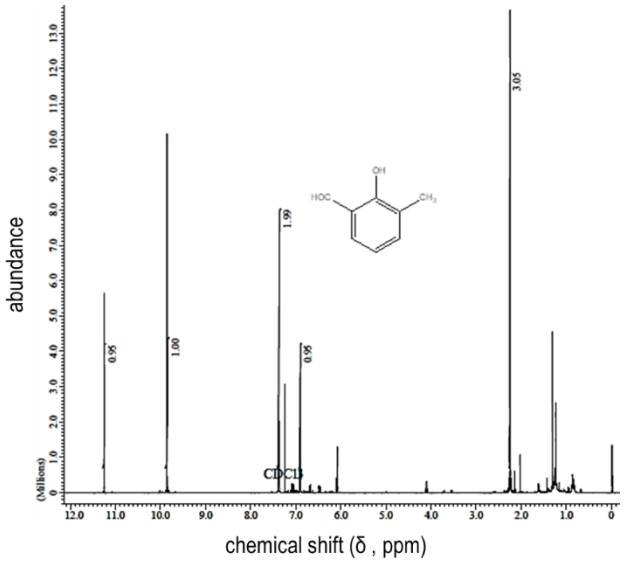


**Figure 3.** H-NMR spectra of 2-hydroxy-3-methylbenzaldehyde

The 2-hydroxy-3-methylbenzaldehyde compound (H-NMR, 500 MHz, $CDCl_3$, δ ppm) =, 11.2547 (s, 1H); 9.852 (s, 1H); 7.363-7.382 (m, 2H); 6.919-6.904 (t, 1H, J = 7.5 Hz); 2.232 (s, 3H).

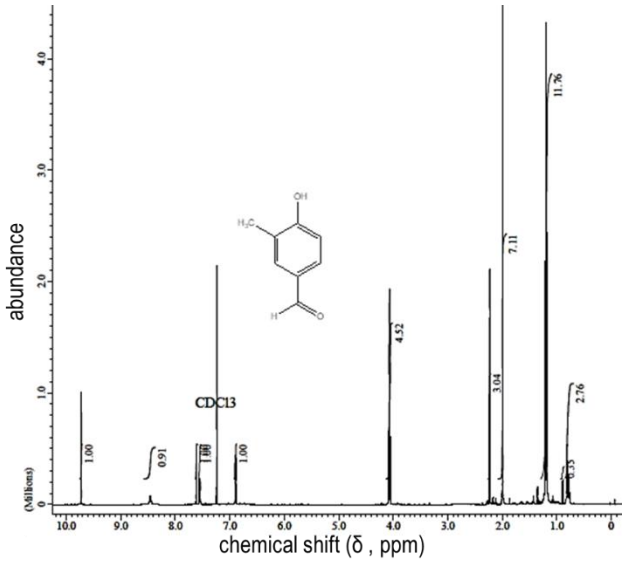


**Figure 4.** H-NMR Spectra of 4-hydroxy-3-methylbenzaldehyde

The 4-hydroxy-3-methylbenzaldehyde compound (H-NMR, 500 MHz, $CDCl_3$, ppm) = δ 9.726 (s,1H); 8.457 (*broad*,1H); 7.562-7.541 (d, 2H, J=8Hz); 7.612 (s,1H); 6.886-6.903 (d,1H, J=8.5 Hz); 2.233 (s,3H).

Furthermore, the compound of 4-hydroxy-3-methylbenzaldehyde was identified using FTIR to determine the functional group in the compound. FTIR spectra of 4-hydroxy-3-methylbenzaldehyde is presented in Fig. 5. The presence of aldehyde groups at wave numbers 1469.65 $cm^{-1}$, 1662.52 $cm^{-1}$ that indicated the vibration of C=O group and the vibration of aromatic compound at wave number 3058.88 $cm^{-1}$.

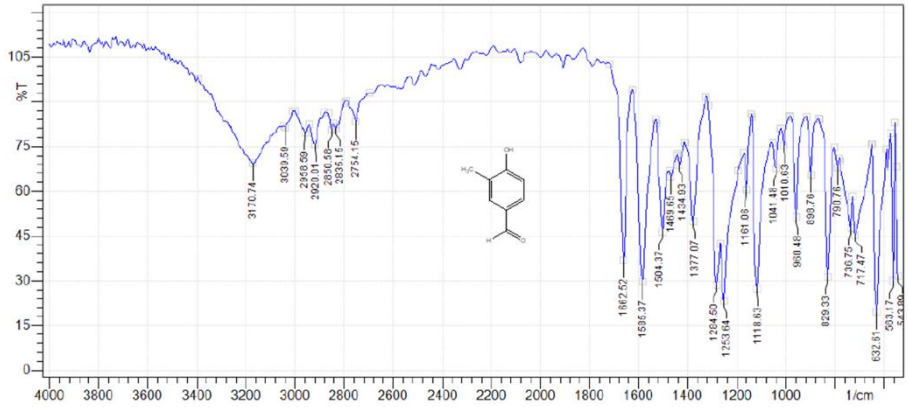


**Figure 5.** FTIR spectra of 4-hydroxy-3-methybenzaldehyde

*3.2. Synthesis of 3-acetylcoumarin compound*

Synthesis of 3-acetylcoumarin compound was done by reacting salicylaldehyde with ethylacetoacetate as reagents and piperidine as catalyst [3]. Reaction of the formation of 3-acetylcoumarin compound is presented in Fig. 6. Then, the reaction was monitored using thin layer chromatography plates (TLC plates) with the solvent system (ethyl acetate: n-hexane 1:3).

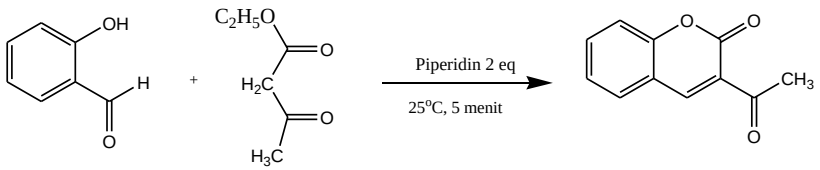


**Figure 6.** Reaction of the formation of 3-acetylcoumarin

When the reaction was monitored, a dominant spot was formed on the TLC plate. Product from this reaction has a yellow solid in about 14.78 g and 95.97% yield. It has a melting point range about 112-115°C. The peak results of UV-Vis spectrophotometry of 3-acetylcoumarine in methanol were seen at wavelengths of 294.2 nm (band I) and 340.2 nm (band II). Then, the results of the H-NMR spectra of 3-acetylcoumarin compound is presented in Fig. 7. The compound of 3-acetylcoumarin (H-NMR, 500 MHz, $CDCl_3$, ppm) = δ 8.492 (s,1H); 7.653-7.619 (m, 2H); 7.360-7.308 (m,2H); 2.708 (s,3H).

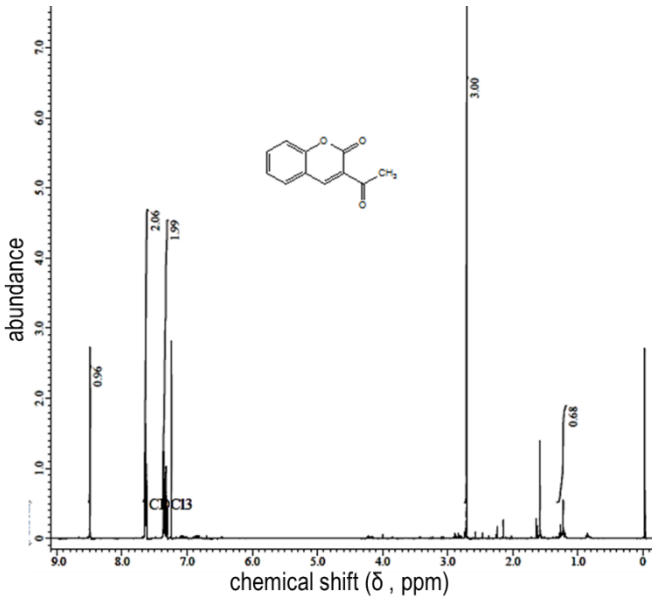


**Figure 7**. H-NMR spectra of 3-acetylcoumarin

*3.3. Synthesis of 3-(3-(4-hydroxy-4-methylphenyl) acryloyl)coumarin compound*

The synthesis of 3-(3 (4-hydroxy-3-methylphenyl)acryloyl) coumarin compound was done by reacting 4-hydroxy-3-methyl benzaldehyde and 3-acetylcoumarin with ethanol solvent and piperidine as catalyst [3, 6]. Reaction was monitored using thin layer chromatography every hour with solvent system (ethyl acetate: n-hexane 1: 3). Reaction of the formation of 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin is presented in Fig. 8

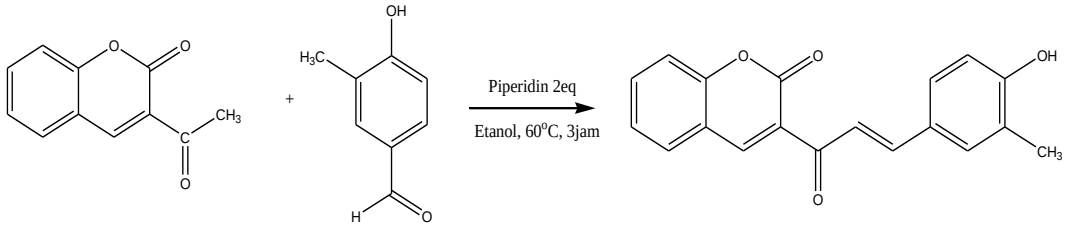


**Figure 8.** Reaction of formation of 3-(3-(4-hydroxy-3-methylphenyl) acryloyl)coumarin.

The TLC profile shows the presence of several compounds, so that they were purified by column chromatography. The eluent system that was used in column chromatography is ethyl acetate: n-hexane. Profiles that were separated are profiles with greenish yellow fluorescence from the TLC results, the greenish yellow profile is the target compound, namely 3- (3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin. The pure product formed a brownish yellow solid in about 0.26 g and 11.66% yield. Then, the product was identified using UV-Vis, LC-MS, and FTIR.

The UV-Vis spectra of 3-(3-(4-hydroxy-3-methylphenyl)acryloyl) coumarin in methanol show maximal wavelength at 278.8 nm with an absorbance 0.49. On the other hand, LC-MS analysis results give the chromatogram and spectra of 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin compound are presented in Fig. 9.

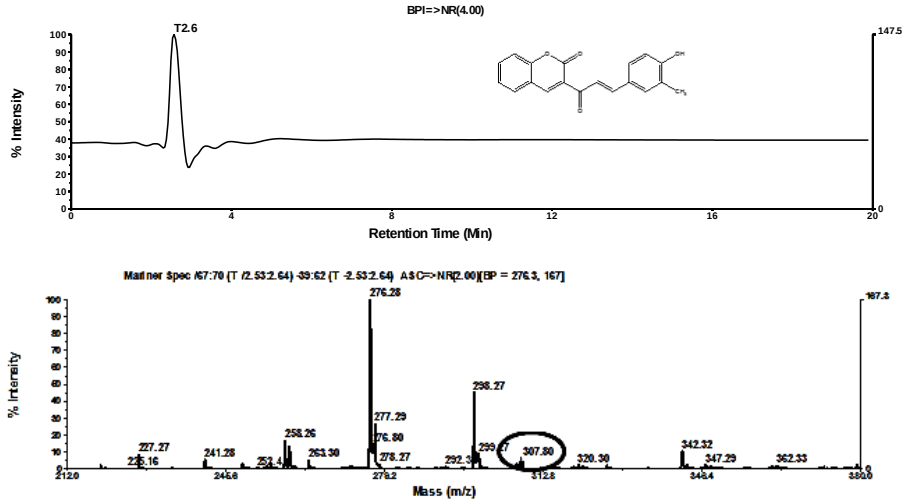


**Figure. 9** Chromatogram and spectra of 3-(3-(4-hydroxy-3 methylphenyl) acryloyl)coumarin.

The 3-(3-(4-hydroxy-3-methylphenyl)acryloyl) coumarin compound with the formula $C_{19}H_{14}O_4$ has molecular weight of 306.30 g/mol. Whereas from LC-MS spectra, the compound has a molecular weight of 307.80 g/mol with $t_R$ (retention time) in about 2.6 minutes with the ionizing compound is $H^+$ (hydrogen) ion. The FTIR spectra of 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin compound is presented in Fig. 10. From the FTIR spectra, it explains that there is a vibration of functional group C=O at wave number 1666.37 $cm^{-1}$, -OH functional group at 3170.74 $cm^{-1}$ and aromatic compound at wave number 3058.88 $cm^{-1}$.

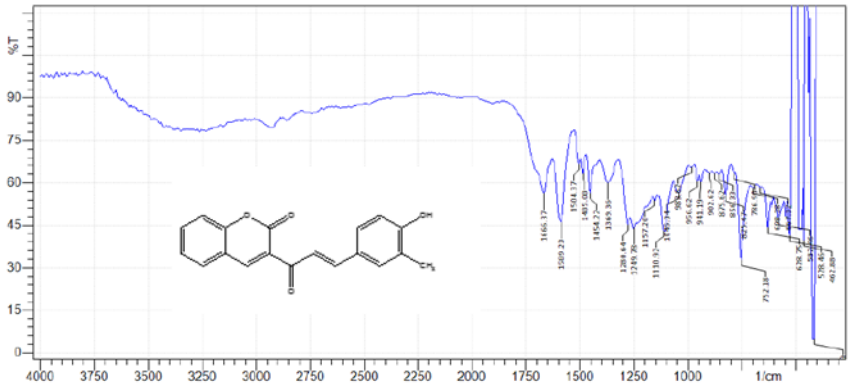


**Figure 10.** FTIR spectra of 3-(3-(4-hydroxy-3-methylphenyl)acryloil)coumarin.

*3.4. Antibacterial activity of 3-(3-(4-hydroxy-3-methylphenyl)acryloyl) coumarin compound.*
Antibacteral activity of 3-(3-(4-hydroxy-3-methylphenyl) acryloyl)coumarin compound was tested against *Escherichia coli* (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria). This antibacterial testing was done by disc diffusion method. It was done by a filter paper was containing a number of active compounds placed on the surface of solid medium which had previously been inoculated with bacteria on the surface of the media. After incubation, inhibitory zone diameter around the disc was used to measure the resistance strength of the active compound to the bacteria [14].

The antibacterial activity of 3-(3-(4-hydroxy-3-methylphenyl) acryloyl)coumarin compound was greater than 3-acetylcoumarin compound. This is related to the difference compound structures. The 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin compound which are one of the derivative compound from the 3-acetylcoumarine and bind to the aldehyde compound. It provides the better antibacterial

activity than 3-acetylcoumarin compound. The presence of methyl and hydroxy groups in the 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin compound can increase their activity antibacterials.

The 3-(3-(4-hydroxy-3-methylphenyl)acryloyl) coumarin compound work as antibacterials by denaturing bacterial cell proteins and damaging the cytoplasmic membrane which can cause leaking of important metabolites and activate the bacterial enzyme system. This damage allows nucleotides and amino acids to exit the cell and prevent the entry of active ingredients into the cell. This condition can cause bacterial death. Antibacterial activity test results of compounds are presented in table 1.

**Table 1**. Inhibitory zone diameter antibacterial activity.

| Compounds (2000 ppm) | *E.coli* (mm) | *S.aureus* (mm) |
|---|---|---|
| 3-acethylcoumarin | 3.50 | 2.80 |
| 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin | 5.46 | 3.38 |
| Amoxicilin (+) | 10.26 | 8.72 |

## 4. Conclusion

Based on the results of this research, it can be concluded that The 3-(3-(4-hydroxy-4-methylphenyl)acryloyl)coumarin compound is formed brownish yellow solid in about 0.26 g and 11.66% yield. From the LC-MS spectra of 3-(3-(4-hydroxy-3-methylphenyl)acryloyl)coumarin compound give a molecular weight in about 307.80 g/mol (M+1). The 3-(3-(4-hydroxy-4-methylphenyl)acryloyl)coumarin in 2000 ppm concentration showed antibacterial activity against *Escherichia coli* (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria). Then, inhibitory zone diameter of the compound is 5.46 mm approximately in *E.coli* bacteria, whereas in *S.aureus* bacteria in about 3.30 mm

## Acknowledgement

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