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Synthesis of resorcinic acid and its *Staphylococcus aureus* antibacterial activity

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Abstract

This study was carried out with an objective to synthesis and investigate the antibacterial potentials of resorcinic acid. The aim of the study is to synthesis and assess the antimicrobial activity and to determine the zone of inhibition of resorcinic acid on *Staphylococcus aureus* bacterial. Today many infectious diseases are common. All of the diseases are caused by agents such as viruses or bacteria which are pathogenic and *Staphylococcus aureus* is one of the pathogenic bacteria. In addition, many antibiotics are not able to work properly because of the resistance of bacteria against the exciting antibiotics. Therefore, research to discover the new anti-bacterial compounds is important to do. The synthesis of resorcinic acid was conducted by reacting resorcinol and carbon dioxide. The characterization of the target compounds was performed by IR and MS spectrometers. The growth of the tested bacteria was observed using a colony counter to see the diameter of the resistance which was caused by the test solution. The antibacterial activity test indicated that resorcinic acid had the potential as an antibacterial against *Staphylococcus aureus.* The activity *was* known from its inhibition zone. At the concentration of 100 ppm, resorcinic acid solution showed an inhibitory diameter of 17.4 mm and amoxicillin antibiotic showed of 16.6 mm. It is mean the resorsinic acid can be used as the potential antibiotic agent.

Keywords: *Synthesis; antibacterial; resorcinic acid; characterization and inhibition zone*

1. Introduction

Infectious diseases are becoming a major cause of human and animal mortality and morbidity. This is further aggravated by the rapid development of multi-drug resistance, limited antibacterial spectrum and adverse effects of available antimicrobial agents [1]. Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance [2]. The emergence of this resistance caused by bacteria can adapt to the presence of antibiotics in clinical concentration and also can be caused by the wrong usage of antibiotic by patient. Various studies discovered that 40-62 % caused by usage of antibiotic for the diseases that not require antibiotics [3]. The resistance of microorganisms to antibiotics is a critical and dangerous medical problem [8]. Some antibiotics that have been resistant as reported [4,5] are chloramphenicol (*P.aeruginosa, K. pneumoniae, E. coli, S. typhimurium, V. cholerae*), macrolides (*Streptococcus pneumoniae, Enterococcus sps, Bacteroides sps, Pseudomonas sps* and Enterobacteriaceae), tetracyclines (*S. aureus, E. coli, A. baumannii, S. typhimurium*), aminoglycosides (*E. coli, P. aeruginosa, A. baumannii*) and also beta-lactams (*H. influenzae, P. aeruginosa, A. baumannii*). In addition, there was increasing of 440,000 new cases due to multidrug-resistant tuberculosis (MDR-TB) each year which causes at least 150,000 cases of death each year. Indonesia ranked eighth out of 27 high MDR load countries (WHO, 2009). There is two ways to develop new antibiotics such as; 1) Isolation of the active compounds in medical plants that traditionally used to treat diseases caused by bacterial [6,7] synthesized the groups of compounds that have been known to have antibiotic activity. Economically, the first way is advantageous if the antibacterial content in plans or microorganism is present in large quantities. However, if the antibacterial content is presented in small quantities the second way will be more profitable. The discovery of new synthetic antibacterial agent effective against resistant microorganisms is important for medicinal chemists. Despite the discovery of many natural and synthetic antibiotics, the innovation of new antibacterial will help in solving the emergence of the microorganisms' resistance problem. The synthetic fenol compound derivates such us dihidroxy xantone possess interesting and have antibacterial activities [3]. The weakness of these reseach is the route of dihidroxy xantone synthesis still long with the less of rendemend. In this study, in order to afford structural requirements for organic antibacterial with one step reaction, and evaluated their activity again *Staphylococcus aureus.* Staphylococcus is a group of bacteria that can cause a number of diseases as a result of infection of the tissues of your body. Human pathogenic bacteria include amongst others *Staphylococcus aureus*; a major cause of bacteremia, associated with higher morbidity and mortality compared to other bacteremia-causing pathogens

2. Experimental

2.1. Tools and Materials

Tools that used in this research were: autoclave, petri dishes, incubator, micropipette, inoculation loop, tweezers, aluminum foil, analytical balance, volumetric flask, sterile cotton, gauze, beaker glass, stirring rod, erlenmeyer flask, separating tube, test tube, cylinder, rotary vacuum evaporator, oven, chamber, visible light, UV-Vis and GC-MS spectrophotometer, laminar air flow, and colony counter. The materials needed in this research are resorcinol, potassium hydrogen carbonate, sterile water, carbon dioxide, concentrated hydrochloric acid, decolorising carbon, *Staphylococcus aureus*, Mueller Hinton Agar medium, amoxicillin, beef broth, Agar powder, and anhydrous Na_2SO_4.

2.2. Syntheis of Resorcylic Acid

A solution containing 40 g (0.364 mol) ofresorcinol, 200 g potassium hydrogen carbonate and 400 mL of water wereplaced in a little flask fitted with a reflux conderser and gas inlet tube. Heat gentle on a steam bath for 4 hours, andreflux vigorously over a flame for 30 minutes while passing a rapid stream of carbon dioxide through the solution. Acidity the solution while still hot by adding 180 mL of concentrated hydrochloric acid from a sparatory funnel with a long delivering acid to the botton of the flask. Allow to cool to room temperature, cill in an ice bath and collect the crude resorcilyc acid by filtration with suction. Recrystallise by boiling the crude acid with 180-200 mL of water in the presence of a little decolorising carbon, filter through a hot water funnel and cool in an ice-salt mixture with stirring. Colect and dry the pure resorcilyc acid. The target compounds then was characterized by IR and MS spectrometers.

2.3. Antibacterial Activity Test

2.3.1. Preparation of The Tested Sample

10 mg of the synthesized compound emulsified in 50 µL dimethyl sulfoxide and than diluted in 100 mL water to obtain the concentration of 100 ppm (as the main liquor). Another tested solutions were prepared to be 20 ppm, 40 ppm, 60 ppm, and 80 ppm from the main liquor.

2.3.2. Preparation of Nutrient Agar Medium

350 mL of sterile water, 350 mL of brief broth and 20 gram of agar powder were heated until thickened in a beaker glass while stirring.

2.3.3. Preparation of Mueller Hinton Agar Medium

500 mL of sterile water mixed with 20 grams of instant Mueller Hinton Agar then heated to be thickened and yellow in a beaker glass while stirring.

2.3.4. Rejuvenation of Tested Bacteria

The bacteria that used as the tested bacteria were inoculated in a 5 mL Nutrient Agar medium in the test tube using the ose needle, at 37ºC for 24 hours, using a scratch method.

2.3.5. Preparation of Comparative Solution

10.0 mg of amoxicillin antibiotic was dissolved adequately in sterile water up to 100 mL to reached a concentration of 100,0 ppm. This solution was used as a positive control.

2.4. Antibacterial Activity Testing Using Agar Diffusion Method (Sumuran)

Bacterial resistance was seen from the diameter of inhibitory zone. 10 mL of Mueller Hinton Medium poured on to sterile petri dishes and allowed to be freeze as the basic layer. After that, 5 mL of the rather cold Mueller Hinton Agar Medium with temperature 45-48 °C mixed well with bacteria up to 6 mL and homogenized. Then poured over the base layer of the medium and distributed evenly using a sterile speader (pour plate method). Furthermore, the incisions were placed on the surface of the medium and filled with 0.2 mL of comparative solution and test solution. A medium consist of 7 tube that divided into a positive control tube which contain amoxicillin, 5 tube which contain difference concentration of methanol extract of cinnamaldehyde and a negative control tube which only contain of sterile water.

The incisions were incubated at temperature of 37 °C for 24 hours. Then the diameter of inhibitory zone or the space that not overgrown by bacterial calculated by using colony counter.

3. Result and discussion

3.1. *Characterisation of Target Compound*

The resorcilyc acid was obtained as a white solid, melting point 169 °C and 67,5 % yield. The synthesized resorcylic acid was characterized by IR, and mass spectrometers. The IR spectrum showed strong absorptions at 3012 and 1642 cm^{-1} indicated the presence of the hydroxyl and carbonyl group of resorcilyc acid, respectively.

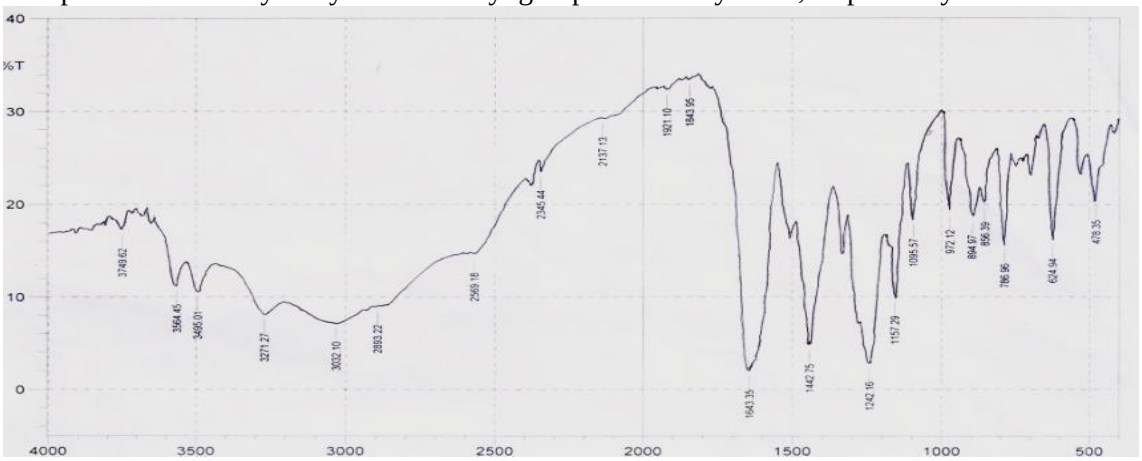

Figure 1: Infra red spectrum of resorcilyc acid

The analysis using Mass Spectrophotometer showed that the target compound was a single component and gave a molecular weight of 154 with the base peak of 136 m/z which resulted by H_2O released, whereas the peak of 108 resulted by the released of C=O group. All of fragmentations given were identical to resorcilyc structure. The result of mass spectrophotometer analysis shown in Figure 2.

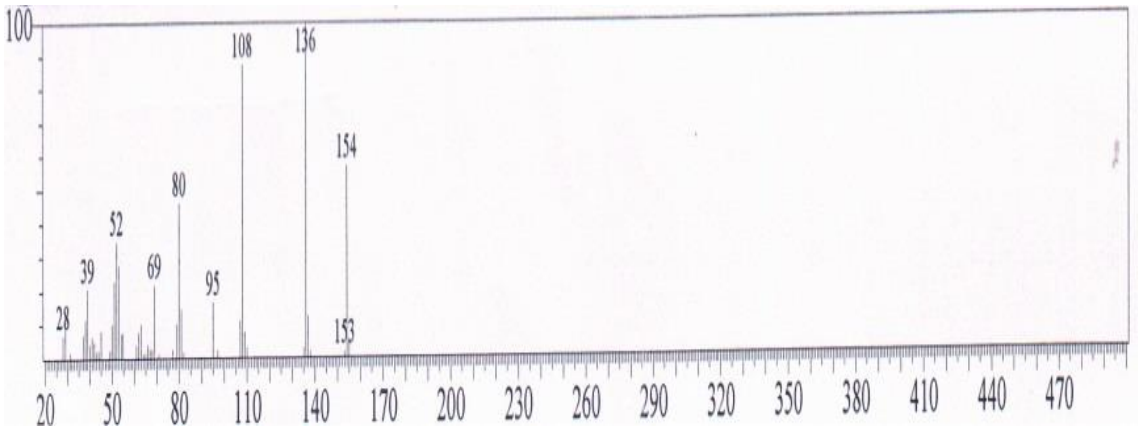

Figure 2: Mass Spectrum of resorcilyc acid

3.2. Antibacterial Activity Test of Resorcilyc Acid

The method used in antibacterial activity test of resorcilyc acid was agar diffusion. In this method, the tested bacteria was bred in medium growth of bacteria then into each medium included various concentration tubes of resorcilyc acid solution which were 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm.

Result of antibacterial activity test of resorcilyc acid that synthesized from the resorcinol and carbondioxide against the growth of Staphylococcus *aureus* listed in Table 1.

Table 1: The Inhibitory Zone Diameter of against *Staphylococcus aureus* (Caption: K^+ is positive control and K^- is negative control)

No	Treatment	Diameter of Inhibitory Zone (mm)			Total	Average
		I	II	III		
1	20 ppm	13.5	13.3	13.4	40.2	13.30
2	40 ppm	14.4	14.6	14.2	43.2	14.40
3	60 ppm	15.6	15.6	15.6	46.8	15.60
4	80 ppm	16.4	16.2	16.3	48.9	16.30
5	100 ppm	17.6	17.4	17.2	52.2	17.40
6	K+	18.2	17.0	14.8	49.8	16.6
7	K-	-	-	-	-	-

The data in Table 1 showed that the resorcilyc acid solution has an inhibitory effect on the growth of *Staphylococcus aureus* as indicated by the mean diameter of different inhibitory zones in each treatment. The increased concentration was in line with the increased of inhibitory zone diameter. According to Davis and Stout (1971), if the diameter of the inhibitory area is 5 mm or less, the inhibiting activity is categorized as weak, 6-10 is categorized as moderate, 11-19 mm is categorized as strong, and 20 mm or more is categorized as very strong. This research revealed that resorcilyc acid provided a strong inhibitory activity because it had inhibitory zone diameter between 11 and 19 mm at concentration of 100 ppm. Amoxicillin was used as a comparative solution because amoxicillin proved 80% resistance to *Escherichia coli* (Krisnaningsih, 2005). Based to the result in the Table 1, inhibitory zone diameter of amoxicillin was 16.6 mm. in other word it was less than inhibitory zone diameter of resorcilyc acid. The higher concentration caused the greater released of antimicrobials, thus facilitating the penetration of the compound into the cell. But the concentration of 100 ppm of resorcilyc acid solution can be quite good in inhibiting bacterial growth compared to the exciting studies. In 2015, Fonkeng et al., declared on his research that at concentration of 409.6 ppm methanol extract of *Ocimum gratissimum* inhibited the growth of *Staphylococcus aureus* with the inhibitory zone diameter of 9.07 mm. Then the result of this research was better than the exciting studies because resorcilyc acid solution of this research provided a considerable inhibitory than the tested solution of the exciting studies. Parekh (2006) also stated that a tested solution provided a potential as an antibacterial if it had the standard inhibition of 14 mm. According to the statement, can be conclude that concentration of 100 pm resorcilyc acid had the potential as an antibacterial with inhibitory of 15.4 against *Staphylococcus aureus.*

4. Conclusion

Resorcilyc acid compounds can be synthesized from resorcinol by using carboxylation method. The product was obtained as a white solid, melting point 169 °C and 67.5 % yield. Mass Spectrophotometer characterization denoted the resorcilyc acid molecular weight of 154. Tested solution of resorcilyc acid with the concentration of 100 ppm provided the potential as an anti-bacterial in inhibitory zone diameter of 15.40 mm against *Staphylococcus aureus*, whereas amoxicillin as the compared solution was 16.60 ppm.

References

[1] J.H. Doughari, B. Okafor, East Cent. African J. Pharm. Sci. 10 (2007) 17–21.

[2] Suwandi, Cermin Dunia Kedokt. 74 (1992) 46–49.

[3] Jumina, Teknologi Produksi 2,3,6,7-Tetrahidroksi-xanton dari Resorsinol dan Karbon Dioksida serta Uji Aktivitasnya sebagai Antiobik Berspektrum Luas, Yogyakarta, 2015.

[4] S. Rao, Antibiotik and Antibiotik Resistance, (2012). http://microrao.com.

[5] C. Dedhia, J.R. Chunduri, Biological Refining : Novel Technique to Reduce the Rancidity of used Edible Oil Biological Refining : Novel Technique to Reduce the Rancidity of used Edible Oil, (2015) 13–19.

[6] O.M. Dorobăţ, A. Moisoiu, D. Tălăpan, I Pneumologia. 56 (2007) 7–15.

[7] I.G.M.. Budiana, M.K. Tokan, A. Saputra, J Applied Chem. Sci. 5 (2018) 469-472.