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Zone of Inhibition of Phenol and Lysol Antimetabolites against Growth of *Pseudomonas aeruginosa* (A Literature Study)

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ABSTRACT

Phenols are the oldest antimetabolites that are often used as disinfectants or microbiosides. Phenols are aromatic compounds that have a distinctive odor and include derivatives of benzene (C6H6). Phenol has a germicidal effect that causes protein denaturation and has active substances on the surface (surfactants) so that it can damage cell membrans and control the growth of microbes that can prevent nosocomial infections in hospitals. *Pseudomonas aeruginosa* is a Gram-negative bacteria that can live on medical equipment and other parts of the hospital that can cause post-surgical infections.The aims of research to determine the inhibition of phenol antimetabolites against *Pseudomonas aeruginosa*.Through a literature study of several research results that have been published in scientific journals, it is known that the method and concentration used are very influential in testing the inhibition of phenol antimetabolites on the growth of *Pseudomonas aeruginosa* bacteria. The results of several literature searches state that phenol antimetabolites are only able to inhibit the growth of *Pseudomonas aeruginosa* with a weak strength of 4±0,5 - 11 mm. In Lysol which is a phenol antimetabolite that reacts with halogens, there is a large difference in the zone of strong (sensitive) inhibition, which is influenced by a large concentration with the greatest inhibitory strength at concentrations of 75% and 100%, with a diameter of 26 mm. From the results of research that has been done it can be concluded that, phenol antimetabolites can inhibit *Pseudomonas aeruginosa*.

KEYWORDS: Phenol antimetabolite , Lysol, *Pseudomonas aeruginosa*

I. INTRODUCTION

Disinfectants are chemicals used to kill microorganisms (antimicrobial) can remove 60% - 90% of microorganisms. [1]Disinfectants are used extensively in hospitals and other health care centers to control microbial growth as well as an important part of infection control practices and help prevent nosocomial infections [2]

An ideal disinfectant is one that has broad-spectrum antimicrobial activity at low concentrations, must be soluble in water or other solvents to the concentration required to be used effectively. Disinfectants must also be stable, non-toxic to humans, active at room temperature, not rust and discolored, able to remove odors, have the ability to act as detergents or cleaners, and be available in adequate quantities at affordable prices [3]. [4], one of the disinfectants that has ideal microbial activity is phenol. Strengthened by [1] and [5]phenol is an effective disinfectant in killing germs. Phenol as an antimicrobial causes protein denaturation and has surface active substances (surfactants) so that it can precipitate cellular proteins and damage cell membranes [6]The specialty of Phenol can be used as a standard of comparison to determine the effectiveness of a disinfectant, because phenol is the oldest disinfectant whose strength has [16] the phenol concentration used for antiseptics is 0.5% -1%, while the concentration used for disinfectants is 5%.

To test the effectiveness of phenol as an anti-microbial it is necessary to test the phenol coefficient. This test was carried out to compare a disinfectant with standard phenol killing power under the same test conditions [3]. Phenol coefficient less than 1 indicates that the antimicrobial agent is less effective than phenol. Conversely, if the phenol coefficient is more than 1, it means that the antimicrobial agent is more effective than phenol.

Researchers conducted a literatur study about the phenol coefficient using the test bacteria *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is one of the most frequently isolated gram-negative bacteria from patients treated in the Intensive Care Unit (ICU) room [7]. The *Pseudomonas aeruginosa* group lives under normal host conditions and

then acts as a saprophyte, but can be a pathogen if the host's defenses are abnormal. [8] *Pseudomonas aeruginosa* is an opportunistic pathogen that can cause invasive conditions in critically ill patients and patients who have very low levels of immunity. Generally, these germs are often found as a cause of nosocomial infections in hospitals [9]. *Pseudomonas aeruginosa* can live on medical equipment and other parts of the hospital, making it easy to infect patients with reduced immunity [7]. According to research conducted by [5]determining the phenol coefficient of floor cleaners containing 2.5% pine oil against *Pseudomonas aeruginosa* bacteria, it was obtained that disinfectants containing phenol antimetabolites were still effective in killing *Pseudomonas aeruginosa* bacteria, which are gram-negative bacteria that cause the most cases of infection. nosocomial in hospital.

Seeing the importance of a disinfectant containing phenol antimetabolites to inhibit the growth of *Pseudomonas aeruginosa*, the researchers are interested in conducting a literature study on testing the inhibition of phenol antimetabolites on the growth of *Pseudomonas aeruginosa.*

II. METHODS

This type of research is descriptive research using the Literature Study method. The dependent variable of the study was the inhibition of *Pseudomonas aeruginosa* bacteria and the independent variable the concentration of phenol antimetabolites. The research topic related to the ability of phenol antimetabolites to inhibit the growth of *Pseudomonas aeruginosa* bacteria, based on the inhibition zone formed from the concentration of phenol antimetabolites. The data analysis technique used in this study uses the content analysis method. The inhibition zone formed can indicate that an antimicrobial is sensitive, intermediate or resistant to the growth of a microbe [10]. The data sources used come from journals and theses that have topics relevant to research in both international, national and national thesis journals. Data sources are also equipped with references to preliminary test results.

III. RESULT

A. Preliminary Test Results of Phenol Antimetabolite Inhibition Zone Against Pseudomonas aeruginosa

In the preliminary test results of the phenol antimetabolite inhibition zone on the growth of *Pseudomonas aeruginosa* showed the formation of a weak strength at all concentrations as shown in table 1.

Table 1. Preliminary Test Result of Phenol Antimetabolite

No	Crystal Phenol Antimetabolite Concentration	Inhibition zone diameter	test results
1	Positive Control (5% phenol antimetabolites)	11mm	Weak
2	Negative Control	0 mm	No inhibition zone
3	Concentration 1/40 (2.5%)	7 mm	Weak
4	Concentration 1/60 (1.6%)	7 mm	Weak
5	Concentration 1/80 (1.2%)	6 mm	Weak
6	Concentration 1/100 (1%)	6 mm	Weak
7	Concentration 1/120 (0.8%)	6 mm	Weak
8	Concentration 1/140 (0.7%)	6 mm	Weak

The preliminary test results on testing the inhibition of phenol antimetabolites on the growth of the *Pseudomonas aeruginosa bacteria* above, are used as one of the references used as one of the data sources for this literature study.

B. Analysis of The Result of Lterature Study

Preliminary test results of Phenol antimetabolite inhibition zone against *Pseudomonas aeruginosa* with 0.5, Mc. Farland with the sensitivity of the agar plate method using sterile disks that have been soaked in various dilution concentrations of phenol antimetabolite solution for 15 minutes, only formed 29 inhibition zone of weak strength on Muller Hinton Agar medium. The results of the analysis [11]on the 5% phenol antimetabolite coefficient test as a standard can kill *Pseudomonas aeruginosa* bacteria at a concentration of 1/80 with the fastest time at -2.5 minutes and the longest time at 15 minutes. And can kill *Pseudomonas aeruginosa* multi resistant (PAMR) at a concentration of 1/70 with the fastest contact time of -2.5 minutes and the longest contact time of 15 minutes. This was also further proven by [5]who stated that phenol antimetabolites could kill *Pseudomonas aeruginosa* bacteria in the fastest time, namely the 5th minute with a concentration of 1/20 and the longest time was in the 30th minute with a concentration of 1/140.

In a study conducted by [12]phenol antimetabolites, have the ability to kill at a concentration of 1/130 in a contact time of 10 minutes but cannot kill the *Pseudomonas aeruginosa* bacteria in a contact time of 5 minutes.

The results of the analysis of several literature studies can be seen in table 2.

Table 2. Analysis of The Result of Lterature Study

No	**Study Literatur**	**RM**	**RR**			
			FKP	**LKP**	**PC**	**IZ**
1	Sulistiyaningsih (2010) Sensitivity Test of Several Antiseptic Preparations against *P. aeruginosa* and *P. aeruginosa* multi resistant (PAMR) bacteria	Phenol coefficient against *P.aeruginosa*	1/80 minute 2.5	1/80 minute 15	-	-
	Sulistiyaningsih (2010) Sensitivity Test of Several Antiseptic Preparations against *P. aeruginosa* and *P. aeruginosa* multi resistant (PAMR) bacteria	Phenol coefficient against *P.aeruginosa*	1/70 minute 2.5 on PAMR	1/70 minute 15 on PAMR	-	-
2	Rahma (2015) in Determining the Phenol Coefficient of Floor Cleaners Containing 2.5% Pine Oil against *P. aeruginosa* bacteria	Phenol coefficient against *P.aeruginosa*	1/120 minute 5	1/140 minute 30	-	-
3	Sentyaningsih (2019) in determining the phenol coefficient of various disinfectants against *P. aeruginosa*	Phenol coefficient against *P.aeruginosa*	1/30 no more than 5 minutes	1/30 minute 10	-	-
4	Nandini and Sumanthy, 2016 in Antimicrobial Activity of Disinfectants and Comparative Study with Phenol	Koefisien Fenol and Sensitivitas Lempeng Agar	-	-	PC on lysol is 1.	Diameter 26 mm at 75% concentration
5	Singh, Rani, and Pal, 2014 in Comparative Efficacy Disinfectant Against Routine Lab Bacterial Contaminant	Koefisien Fenol and Sensitivitas Lempeng Agar	-	-	PC on lysol is 6.6	At 0.6% lysol the diameter formed was 26 ± 331.25 mm at 100% concentration and 4 ± 0.5 at 5% phenol.

Description:
RM : Research Methods
RR : Research Result
FKP : Fastest Killing Power
LKP : Longest Killing Power
PC : Phenol coefficient
IZ : Inhibition Zone

IV. DISCUSSION

Weak ability of 5% phenol antimetabolites to inhibit the growth of *Pseudomonas aeruginosa* bacteria, because phenol antimetabolites interact with bacterial cells through an absorption process that involves 30 hydrogen bonds. Phenol antimetabolites at low concentrations are still able to form protein and phenol complexes, with weak bonds and immediately undergo decomposition followed by penetration of phenol into cells and cause protein precipitation and protein denaturation [11]

In the discussion of [5] research, the different test methods between the phenol coefficient method and the diffusion method using MHA media are a possible cause of the differences in the results of the tests carried out, where the phenol coefficient method is the standard test to determine the effectiveness of a disinfectant.

Whereas in another study conducted by [13] using a modified method from Kirby Bauer it was found that 5% phenol antimetabolites could only form zones of inhibition of weak strength and were not stronger than the zones of inhibition formed on Lysol 0 .6% with a dilution concentration of 100% which has a phenol coefficient value

of 6.6. This is because Lysol can form very strong/sensitive zones of inhibition.

In a [14]using the disk diffusion inhibition zone test method, it was found that Lysol, which had a phenol coefficient value of 1, was able to inhibit the growth of *Pseudomonas aeruginosa* bacteria with very strong abilities at a dilution concentration of 75%, but its inhibitory strength decreased to medium/intermediate at 100% dilution concentration. Furthermore, according to [14], the level of concentration of Lysol is not directly proportional to the diameter of the resulting inhibition zone. Concentration of 75%, the inhibition zone reached 26 mm, while at 100% concentration, the inhibition zone reached 20 mm. [10], the large Lysol inhibition zone at a concentration of 75% has a strong or sensitive *Pseudomonas aeruginosa* growth inhibition response, while the large inhibition zone at 100% Lysol concentration has a medium or intermediate growth inhibition response.

Several factors can affect the diameter of the inhibition zone for bacterial growth, including the turbidity of the bacterial suspension. If the suspension is less turbid, then the diameter of the inhibition zone will be smaller. Incubation temperature can also be a factor affecting the diameter of the zone of inhibition of bacterial growth. Incubation was carried out at 35°C. Temperatures that are less than 35oC can cause a larger diameter of the inhibition zone. The thickness of the agar media can also be one of the factors that affect the diameter of the bacterial growth inhibition zone. The thickness of the effective agar medium is about 4 mm, if less than 4 mm, diffusion will be slow [15]The large irregularity of the inhibition zone for the growth of the test bacteria can also be caused by the unequal disk drying time. Discs with a long drying time, when placed on a bacterial seeding medium will form a small inhibition zone, this zone is formed from the extract that diffuses on the disc into the agar medium. On disks with short drying time, it quickly diffuses into the agar medium to form a larger inhibition zone [16].

The active compound is one of the environmental factors affect the growth of bacteria, providing different effects in inhibiting growth such as nutrition, temperature, pH, and humidity. The ability of the material to inhibit bacteria can also be influenced by the nature of the bacterial cell wall itself [17].

Pseudomonas aeruginosa is the fourth most common nosocomial pathogen isolated from all hospital-acquired infections [18]; [19].These bacteria are Gram-negative bacteria, in the form of coccobacillus with unipolar motility (Soedarto, 2014). cell walls consisting of mucopeptides (peptidoglycan), are usually the target of the enzyme lysozyme [21].

[22]explained that Gram-negative bacteria have a cell wall with a relatively thinner peptidoglycan layer, containing lipopolysaccharide which plays a role in preventing the entry of hydrophobic compounds into the cell membrane, while lipids play a role in preventing the entry of hydrophilic compounds, so that the two layers of the membrane this allows gram-negative bacteria to survive. [14]and [13] that Lysol has a very strong inhibitory ability against *Pseudomonas aeruginosa* bacteria compared to phenol antimetabolites. Lysol contains a mixture of cresol and soap, with the addition of sodium hypochlorite.Classified as halogens such as chlorine can improve the quality of disinfectants and increase phenolic activity [13].

[14]explained that phenol antimetabolites have antifungal and antiviral properties. Its antifungal ability can damage cell membranes and result in leakage of intracellular constituents. Phenol does not affect the transduction of the *Pseudomonas aeruginosa* bacteria and has no effect on the DNA inside the capsid, phenol also only has a weak ability on proteins, unless the treatment is carried out for 20 minutes or more.

In the research results, pure phenol antimetabolites were only able to inhibit *Pseudomonas aeruginosa* bacteria with weak strength at several dilution concentrations of phenol antimetabolites with an inhibition zone of 4 ± 0.5 - 11 mm. However, disinfectants containing phenolic antimetabolites with added halogen content such as Lysol, had the strongest inhibitory ability at a dilution concentration of 75% with an inhibition zone of 26 mm. The results of this study are consistent with the results of a study conducted [13], where 0.6% Lysol was able to form a zone of inhibition of 26 ± 3.25 mm at a concentration of 100%.

V. CONCLUSION

5% phenol antimetabolite, capable of forming a weak strength (resistant) inhibition zone with a diameter of 4 ± 0.5 - 11 mm. Lysol 0.6%. is a phenol antimetabolite reacting with halogen content, forming a strong inhibition zone (sensitive) with a diameter of 26 mm at a dilution concentration of 75% and with a diameter of 26 ± 3.25 at a concentration of 100%.

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