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Bactericidal effects of Extract Bacil Leaves in in-vitro study of Pesudomonas aeruginosa

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Abstract: Pseudomonas aeruginosa is the most common bacterial cause of nosocomial infections. Bacteria become resistant to antibiotics by various mechanisms, including producing enzymes that can damage antibiotics, change intracellular targets from antibiotics and efflux pumps. Basil (Ocimum sanctum L.) is a traditional plant that is usually used as ingredients that contain antibacterial compounds including flavonoids, tannins, alkaloids, eugenol. The aim was to determine the effectiveness of extract basil leaves (Ocimum sanctum L.) for inhibiting and killing the growth of Pseudomonas aeruginosa. We administrated the extract basil leaves (Ocimum sanctum L.) with a concentration of 100%, 50%, and 25% in to the plate contained bacterium Pseudomonas aeruginosa. The result showed that broth dilution for 8 hours and 24 hours there is no inhibition of bacterial growth. And we continued to cultured bacteria for 24 hours. The analysis showed the extract of Ocimum sanctum L has bactericidal effects in 8 hours and 24 hours incubation significantly ($p < 0.05$). However, in 24 hours more effective as a bactericidal in 100% of concentration significantly ($p < 0.05$). From this result, eugenol which is a phenol derivative found in the ethanol extract of basil leaves has the effect of damaging cell membranes. Phenol bonding with bacterial cell walls can disrupt the permeability of transport cell membranes so that the bacteria will be disrupted and die.The extract of basil leaves effective as a bactericidal to Pseudomonas aeruginosa.

Keywords: Pseudomonas aeruginosa, Bactericidal, Extract basil leaves (Ocimum sanctum L.)

1. Introduction

Pseudomonas aeruginosa is the most common bacterial cause of infection in a hospital environment. The incidence of nosocomial infections in the world caused by the bacterium Pseudomonas aeruginosa is around 10-15%. A study conducted by WHO showed that around 8.7% of 55 hospitals from 14 countries from Europe, Middle East, Southeast Asia and the Pacific showed a nosocomial infection. The prevalence of most nosocomial infections in the Eastern Mediterranean and Southeast Asia is 11.8% and 10.0% while those in Europe and the Western Pacific are 7.7% and 9.0% respectively. In Indonesia, namely in the Adam Malik Haji Central Hospital in Medan, nosocomial infections are quite high at 6-16% (Nugraheni et al, 2002). Based on data from The National Healthcare Safety Network that Pseudomonas aeruginosa was ranked first most after Staphylococcus aureus and Acinetobacter baumannii. Infection caused by Pseudomonas aeruginosa is difficult to treat. This is because more strains are resistant to several antibiotics (Multidrug Resistance). Bacteria become resistant to antibiotics by various mechanisms, including by producing enzymes that can damage antibiotics, change intracellular targets from antibiotics and efflux pumps (Gunawan et al., 2009). Karvanen (2013) has conducted a study that colistine is effective against gram negative bacteria including Pseudomonas aeruginosa. Colistine is currently used by inhalation, oral and parenteral, but this drug is difficult to obtain in the Indonesian region and drug preparations are often used for inhalation treatment with the help of a nebulizer (Koomanachai, 2007). Basil (Ocimum sanctum L.) thrives in tropical and subtropical regions, one of them in Indonesia. Basil leaves (Ocimum sanctum L.) contain compounds that are antibacterial including flavonoids, tannins, alkaloids, eugenol and others (Angelina, 2015).

2. Research Methodology

Basil leaves extractions

We was purchase 2 kg of the basil leaves from Keputran traditional market in Surabaya. Then we extract the basil leaves to laboratory biochemristry Hang Tuah University Surabaya for 5 days.

Bacterial strains

We was colleted the bacterial strains Pseudomonas aeruginosa ATCC 27853 provided by the Balai Besar Laboratorium Kesehatan (BBLK) Surabaya. The inoculum suspension was obtained by taking colonies from 24 hours cultures. The colonies were suspended in sterile 0.9% aqueous solution of NaCl. The density was adjusted to the turbidity of a 0.5 McFarland Standard (108 colony forming unit [CFU]/mL). Broth dilution assay Broth dilution assay is one of the most basic methods for antimicrobial susceptibility testing. This procedure involves doubling dilution preparations of antimicrobial agents with concentrations of 25%, 50%, 100% in liquid growth media, then inoculated with microbes in the oculus prepared in the same medium after standardized microbial suspension dilution and adjusted to the McFarland 0,5. After mixing, the tubes in grafting are incubated (mostly without agitation) under conditions of 37 ° C for 24 hours. All the tests were performed in four times.

Determination of MIC

Strains of the Pseudomonas aeruginosa species were chosen for the in vitro MIC and MBC study. Basil leaves extract were investigated for their MIC and MBC against the chosen isolated Pseudomonas aeruginosa strains where 1 ml of the tested extract was used in dilution method with series of 3 tubes containing 1 ml of Mueller Hinton broth to achieve final dilutions of 25%, 50% and 100%. Bacterial inoculums with 0.5 McFarland Standard of the chosen isolated Pseudomonas aeruginosa were inoculated into all 3 dilutions post thorough extract mix. The inoculated tubes were 8 hours and overnight incubated at 37°C. The highest dilution of the tested basil leaves extract to inhibit growth (no turbidity in the tube) was considered as the MIC value of this extract batch against the tested bacterial species.

Determination of MBC

From all tubes showed no visible signs of growth/turbidity (MIC and higher dilutions), loopfuls were inoculated into sterile Mueller Hinton agar plates by streak plate method. The plates were then 8 hours and overnight incubated at 37°C. The least concentration that did not show any growth of tested organisms was considered as the MBC value of the tested extract against the tested bacterial.

Analysis statistics

The values of inhibition and bactericidal are given as mean ± standard deviation (SD). The result were analyzed by Kruskall-Wallis followed by Mann-Whitney Test, using IBM SPSS Statistics 24 for windows software. The result if $P< 0.05$ then it is considered statistically significant.

3. Result and Discussion

The Effects Of Extract Basil Leaves In Minimum Inhibitory Concentration

The antibacterial activity of the basil leaves extract tested against Pseudomonas aeruginosa no any bacterial inhibition in all concentration and all incubation. There is no growth in these bacteria indicated by still appearing clear at all concentrations except positive controls containing bacteria and antibiotics and also negative controls which only contain the bacterium pseudomonas aeruginosa according to what is shown in pictures A and B. This occurs at all times of treatment which are 8 hours and 24 hours incubation.

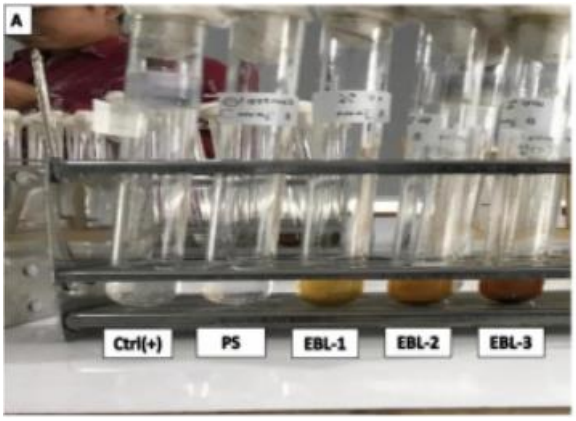

Figure A: Result of the effect of extract basil leaves in minimum inhibitory consentration with broth methods for 8 hours. Ctrl (+); positive controls contain bacteria Pseudomonas aeruginosa and antibiotic, PS; negative controls contain bacteria Pseudomonas aeruginosa only, EBL-1; extract basil leaves with 25% concentration, EBL-

Figure B: Result of the effect of extract basil leaves in minimum inhibitory consentration with broth dilution for 24 hours. Ctrl (+); positive controls contain bacteria Pseudomonas aeruginosa and antibiotic, PS; negative controls contain bacteria Pseudomonas aeruginosa only, EBL-1; extract basil leaves with 25% concentration, EBL-2; extract basil leaves with 50% concentration, EBL-3;

The Effects Of Extract Basil Leaves In Minimum Bactericidal Concentration

Then we were inoculated, the result show any growth in all concentration with 8 hours and 24 hours incubation, but the least growth in 24 hours. This bacterial growth is seen by the presence of bacterial colonies on the plate shown in figures C and D. Then these results are supported by statistical analysis showed the extract of basil leaves has bactericidal effects in 8 hours and 24 hours incubation significantly ($p < 0.05$). However, in 24 hours more effective as a bactericidal in 100% of oconcentration significantly ($p < 0.05$).

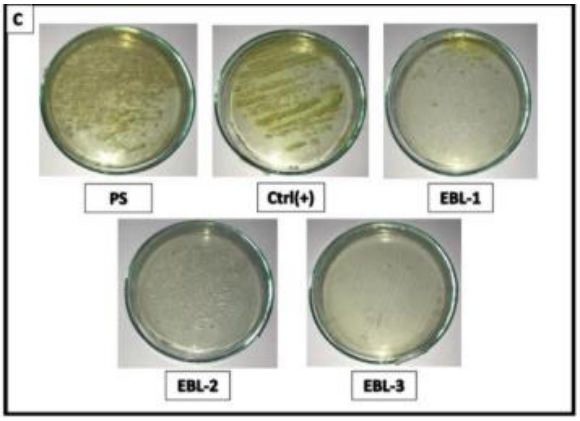

Figure A: Result of the effect of extract basil leaves in minimum bactericidal consentration with broth methods for 8 hours. Ctrl (+); positive controls contain bacteria Pseudomonas aeruginosa and antibiotic, PS; negative controls contain bacteria Pseudomonas aeruginosa only, EBL-1; extract basil leaves with 25 concentration, EBL-2; extract basil leaves with 50% concentration, EBL-3; extract basil leaves with 100% concentration.2; extract basil leaves with 50% concentration, EBL3; extract basil leaves with 100% concentration

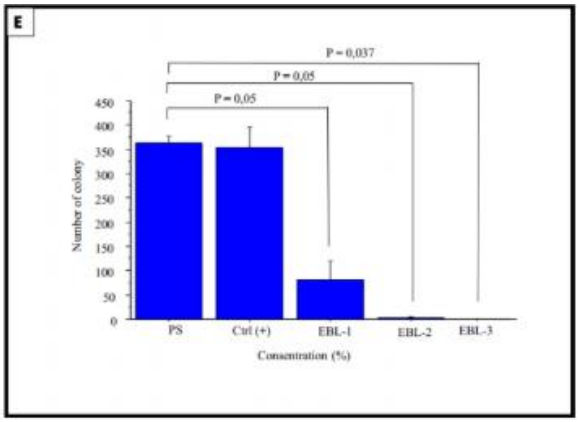

Figure E: Result statisctic analysed of the effect of extract basil leaves in minimum bactericidal consentration with broth methods for 8 hours. Ctrl (+); positive controls contain bacteria Pseudomonas aeruginosa and antibiotic, PS; negative controls contain bacteria Pseudomonas aeruginosa only, EBL-1; extract basil leaves with 25% concentration, EBL-2; extract basil **l**eaves with 50% concentration, EBL-3; extract basil leaves with 100% concentration.

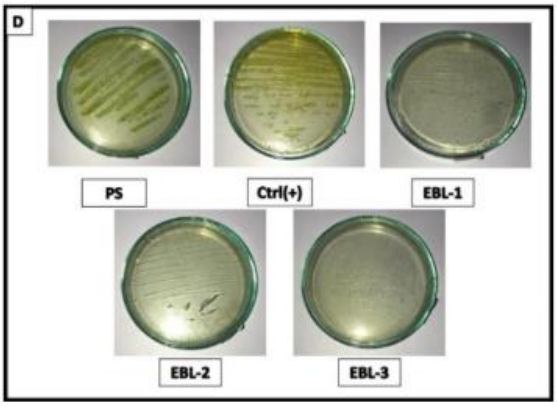

Figure D: Result of the effect of extract basil leaves in minimum bactericidal consentration with broth methods for 24 hours. Ctrl (+); positive controls contain bacteria Pseudomonas aeruginosa and antibiotic, PS; negative controls contain bacteria Pseudomonas aeruginosa only, EBL-1; extract basil leaves with 25% concentration, EBL-2; extract basil leaves with 50% concentration, EBL-3; extract basil leaves with 100% concentration

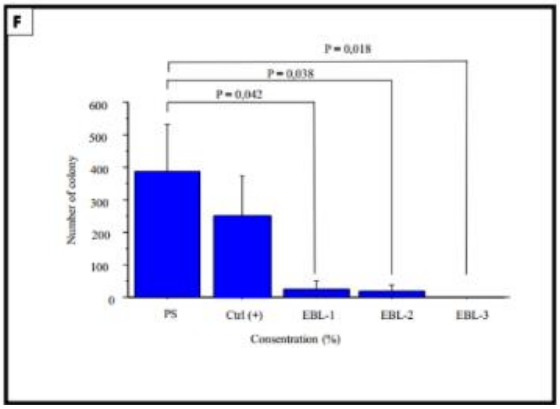

Figure F: Result statisctic analysed of the effect of extract basil leaves in minimum bactericidal consentration with broth methods for 24 hours. Ctrl (+); positive controls contain bacteria Pseudomonas aeruginosa and antibiotic, PS; negative controls contain bacteria Pseudomonas aeruginosa only, EBL-1; extract basil leaves with 25% concentration, EBL-2; extract basil leaves with 50% concentration, EBL-3; extract basil leaves with 100% concentration.

In the results of this study it was found that in broth dilution with 8 hours incubation there was no bacterial growth at all concentrations. Then for 24 hours incubation there was also no bacterial growth at all concentrations. This is in accordance with previous studies that the minimum inhibitory concentration of extract basil leaves on the bacteria Propionibacterium acnes occurred at a concentration of 2% (Hapsari, 2018).

Then replanting the plate containing Mueller Hinton agar media so that at all concentrations. The results showed that at 8 hours incubation bacterial growth was still shown in Figures C and E. This happened because it had not reached the supposed incubation period of 18-24 hours (CLSI, 2013). But this growth occurs in negative controls which only contain bacteria Pseudomonas aeruginosa, positive controls that contain bacteria with antibiotics and at a concentration of 25%. While at a concentration of 50% and 100% the growth of bacteria has begun to be invisible or minimum. Then for the 24-hour incubation, bacterial growth was still shown in Figure D and F. This growth occurred in the negative control which only contained bacteria Pseudomonas aeruginosa, positive control containing bacteria with antibiotics, concentration of 25% and concentration of 50%. Whereas at a concentration of 100% bacterial growth does not occur.

From the results of the study which showed the ethanol extract of basil leaves (Ocimum sanctum L.) was effective against the bacteria Pseudomonas aeruginosa in all concentration. Then it can also kill the growth of Pseudomonas aeruginosa at various different concentrations. Same as previous research by Mishra and Mishra (2011) found basil leaves extract effective against both gram positive and gram negative bacteria. This ethanol extract of basil leaves (Ocinum sanctum L.) is effective because it has antibacterial properties such as alkaloids, tannins, eugenol and flavonoids. Gram bacteria positive only consists of two layers namely lipopolysaccharide and protein with content lipids by 1% - 4%. Whereas in bacteria gram negative has three layers of peptidoglycan which consists of phospholipid, protein, and lipopolysaccharide with a lipid content of 11% - 22%. This content affects the cell wall of the bacterium Pseudomonas aeruginosa so that the bacterial cell wall is damaged and inhibits the growth of the bacteria (Jawezt, et al., 2001).

The antibacterial relationship of the ethanol extract of basil leaves (Ocimum sanctum L.) has one of the competencies namely eugenol. Where eugenol is an antibacterial compound which is a derivative of the class of phenol composition which has the effect of damaging cell membranes. The bond between phenol and bacterial cell walls will activate the permeability of cell membranes and the transportation process, so that bacterial cells will lose cations and macromolecules which cause increased cell growth and death. Then in high concentrations it will cause protein freezing so that bacterial cells die (Maryati, Ratna, and Triastuti, 2007). These results are in accordance with previous

studies by Hapsari (2018) which used extract (Ocimum basilicum L.) to effectively inhibit and try to repair Propionibacterium acnes bacteria which is a gram positive bacterial. The current study by Rahman (2010) had result the zone diameter of inhibitoin was 17 mm due to the action of methanol extract of O. sanctum leaf against S. aureus and The O. sanctum zone diameter of inhibiting for S. typhi was 6 mm, as has been reported by Joshi et al (2009)

4. Conclusion

The extract of Basil leaves effective as a bactericidal to Pseudomonas aeruginosa

5. Thank You Note

We declare that we have no conflict of interest and i'm sincerely thank to RISTEKDIKTI for support funding this research, Nahdlatul Ulama Surabaya University for support fully our research, and Hotimah Masdan Salim, dr., Ph.D, as a supervisor who is attentive, patient, accompanies and directs the author, Staff of laboratory Nahdlatul Ulama Surabaya University Gardina Riska Pratiwi and Cholilul Rachmansyah then especially to my parents who always give support and motivate in the preparation and complete of this article.