ORIGINAL ARTICLE

Bactericidal Effects of Extract Basil Leaves in In-vitro Study of *Pseudomonas aeruginosa*

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ABSTRACT

Introduction: *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, and eugenol. The aim was to determine the effectiveness of extract basil leaves for inhibiting and killing the growth of Pseudomonas aeruginosa.
Methods: We administrated extract basil leaves with a concentration of 100%, 50%, and 25% in the plate contained bacterium *Pseudomonas aeruginosa*. The result showed that there was no inhibition of bacterial growth in broth dilution for 8 hours and 24 hours. We continued to culture bacteria for 24 hours.
Results: : The analysis showed extract 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 concentration significantly ($p < 0.05$)
Conclusion: From this result, eugenol (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, thus the bacteria will be disrupted and die.

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 are 11.8% and 10.0%, while those in Europe and the Western Pacific are 7.7% and 9.0% respectively. In Indonesia, namely in Adam Malik Haji Central Hospital in Medan, nosocomial infections are quite high at 6-16%.[1]

Based on the data from The National Healthcare Safety Network, *Pseudomonas aeruginosa* was ranked after Staphylococcus aureus and Acinetobacter baumannii. Infection caused by *Pseudomonas aeruginosa* is difficult to treat. This is due to 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.

Karvanen (2013) has conducted a study that showed colistin is effective against gram negative bacteria including *Pseudomonas aeruginosa*. Colistin is currently used by inhalation, oral and parenteral, but this drug is difficult to obtain in Indonesia and drug preparations are often used for inhalation treatment with the help of a nebulizer.[2]

Basil (*Ocimum sanctum* L.) thrives in tropical and subtropical regions, one of them is Indonesia. Basil leaves contain compounds which are antibacterial including flavonoids, tannins, alkaloids, eugenol, and others.[3]

Methods

This study Bacterial strains

We collected *Pseudomonas aeruginosa* ATCC 27853 that was provided by Balai Besar Laboratorium Kesehatan (BBLK) Surabaya. The inoculum suspension was obtained by taking colonies from 24 hours cultures.

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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 involved 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 were incubated (mostly without agitation) under conditions of 37°C for 24 hours. All tests were performed four times.

Determination of MIC

Strains of Pseudomonas aeruginosa 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

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 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 were 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. The results, if showing $p < 0.05$, then it was considered statistically significant.

Results

The Effects of Extract Basil Leaves in Minimum Inhibitory Concentration

The antibacterial activity of the basil leaves extract tested against Pseudomonas aeruginosa showed no any bacterial inhibition in all concentration and all incubation. There was no growth in the bacteria indicated by the clear appearance at all concentrations, except positive controls containing bacteria and antibiotics, and also negative controls, which only contained the bacterium Pseudomonas aeruginosa according to what is shown in Pictures A and B. This occurred at all times of treatment which were 8 hours and 24 hours incubation.

The Effects of Extract Basil Leaves in Minimum Bactericidal Concentration

Then the bacteria were inoculated, the results showed any growth in all concentration with 8 hours and 24 hours incubation, but the least growth was in 24 hours. This bacterial growth was seen by the presence of bacterial colonies on the plate shown in Figures C and D. Then these results were supported by statistical analysis which showed extract of basil leaves had 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 ($p < 0.05$).

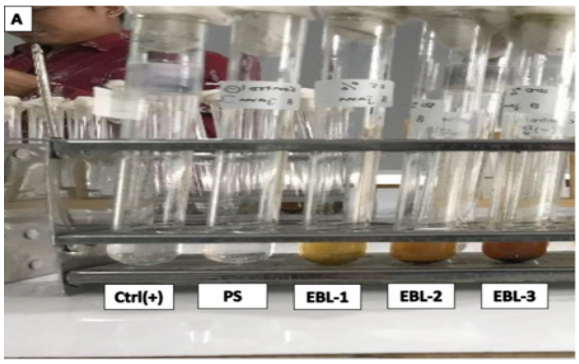

Figure A. Results of the effect of extract basil leaves in minimum inhibitory consentration with broth methods for 8 hours. Ctrl (+); positive controls contained bacteria Pseudomonas aeruginosa and antibiotics, PS; negative controls contained bacteria Pseudomonas aeruginosa only, EBL-1; extract basil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

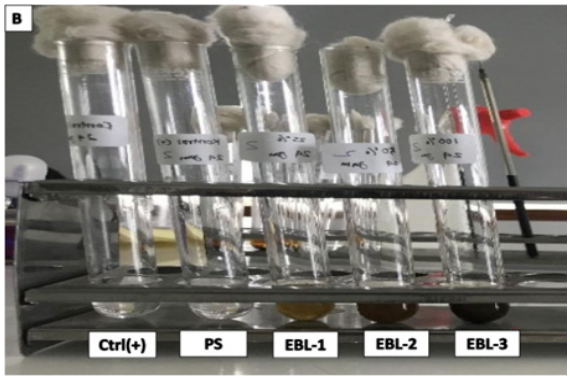

Figure B. Results of the effect of extract basil leaves in minimum inhibitory consentration with broth dilution for 24 hours. Ctrl (+); positive controls contained bacteria Pseudomonas aeruginosa and antibiotics, PS; negative controls contained bacteria Pseudomonas aeruginosa only, EBL-1; extract basil leaves with 25% concentration, EBL-2; extract basil leaves with 50% concentration, EBL-3; extract basil leaves with 100% concentration.

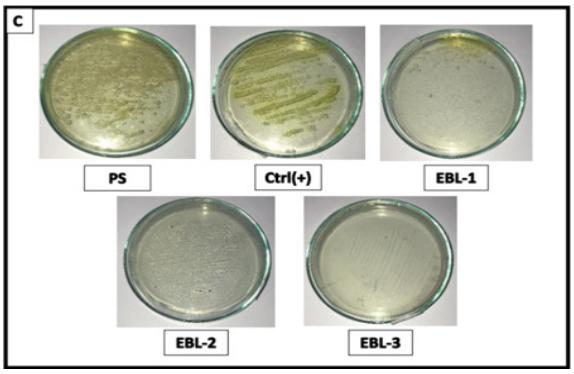

Figure C. Results of the effect of extract basil leaves in minimum bactericidal consentration with broth methods for 8 hours. Ctrl (+); positive controls contained bacteria Pseudomonas aeruginosa and antibiotics, PS; negative controls contained bacteria Pseudomonas aeruginosa only, EBL-1; extract basil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

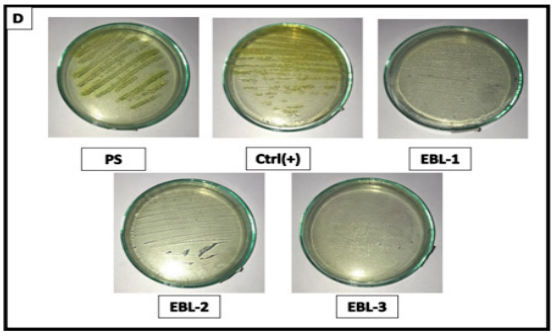

Figure D. Results of the effect of extract basil leaves in minimum bactericidal consentration with broth methods for 24 hours. Ctrl (+); positive controls contained bacteria Pseudomonas aeruginosa and antibiotics, PS; negative controls contained bacteria Pseudomonas aeruginosa only, EBL-1; extract basil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

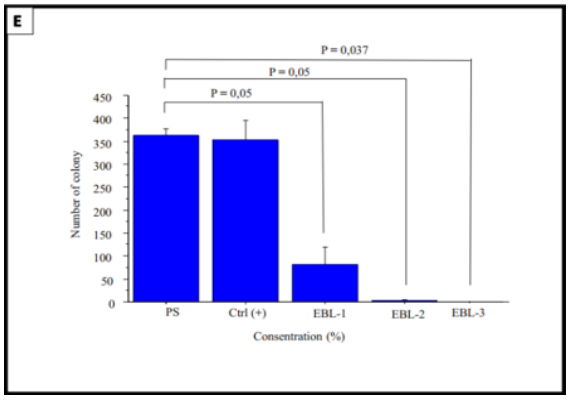

Figure E. Results of statiscticstatistical analysised of the effect of extract basil leaves in minimum bactericidal consentration with broth methods for 8 hours. Ctrl (+); positive controls contained bacteria Pseudomonas aeruginosa and antibiotics, PS; negative controls contained bacteria Pseudomonas 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.

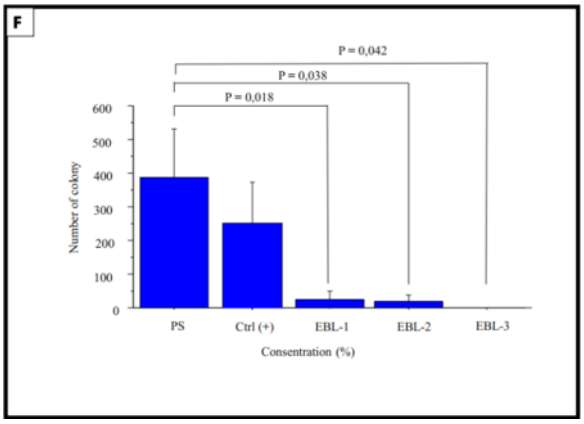

Figure F. Results of statiscticstatistical analysised of the effect of extract basil leaves in minimum bactericidal consentration with broth methods for 24 hours. Ctrl (+); positive controls contained bacteria Pseudomonas aeruginosa and antibiotics, PS; negative controls contained bacteria Pseudomonas 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.

Discussion

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%.[4]

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.[5] Nevertheless, this growth occurred in negative controls which only contained bacteria *Pseudomonas aeruginosa*, positive controls that contained bacteria with antibiotics, and at a concentration of 25%. Meanwhile, at a concentration of 50% and 100%, the growth of bacteria had 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 controls which only contained bacteria *Pseudomonas aeruginosa*, positive controls that contained bacteria with antibiotics, concentration of 25%, and concentration of 50%. Whereas at a concentration of 100%, bacterial growth did not occur.

From the results of the study, it showed that ethanol extract of basil leaves was effective against the bacteria Pseudomonas aeruginosa in all concentration. It could also kill the growth of *Pseudomonas aeruginosa* at different concentrations. This is in line with the previous study by Mishra which found basil leaves extract was effective against both gram positive and gram negative bacteria.6 This ethanol extract of basil leaves was 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 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, thus the bacterial cell wall is damaged and inhibits the growth of the bacteria.[7]

The antibacterial relationship of the ethanol extract of basil leaves has one of the competencies namely eugenol. 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, thus bacterial cells will lose cations and macromolecules which cause increased cell growth and death. In high concentrations, it will cause protein to freeze, thus bacterial cells will die.[8] These results are in accordance with previous studies by Hapsari which used extract basil leaves to effectively inhibit and try to repair Propionibacterium acnes bacteria which is a gram positive bacterial.[4] The current study by Dev showed the zone diameter of inhibition was 17 mm due to the action of methanol extract of basil leaves against *S. aureus* and the zone diameter of inhibiting for *S. typhi* was 6 mm, as reported by Joshi, et al.[9, 10]

Conclusion

The extract of basil leaves is effective as a bactericidal to Pseudomonas aeruginosa.

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Conflict of Interest

The author stated there is no conflict of interest

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