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Research Article

COMBINATION ACTIVITY OF ALOE VERA LEAVES EXTRACT (*Aloe vera* (L.) Burm. F) AND GENTAMICIN AGAINST BACTERIA IN DIABETIC ULCERS

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ABSTRACT

The prevalence of morbidity from diabetic ulcers infection that occurred in Indonesia in 2009 reached 17-23%. Gentamicin sulphate used to treat bacteria in gangrene has shown bacterial resistance. Combining plant extract with antibiotic is one of way to prevent bacterial resistance. The aim of this research was to know the value of MIC (Minimum Inhibitory Concentration) single, combination activity and its effect based on FICI value (Fractional Inhibitory Concentration Index) from combination of *Aloe vera* leaves extract (*Aloe vera* (L.) Burm.f.) and gentamicin sulphate against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Determination of MIC and FICI values using *Kirby-Bauer* disc diffusion method. The concentrations of extract solution were 0.3125; 0.625; 1.25; 2.5; 5; and 10 mg/ml while the concentrations of gentamicin sulphate solution used were 0.25; 0.5; 1; 2; 4; and 8 µg/ml. Combination solution was made with volume ratio 1 : 1 from MIC extract and gentamicin sulphate. The results showed that the value of MIC extract on *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* were 2.5 and 0.625 mg/ml. While the value of MIC gentamicin sulphate in bacteria *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* were 0.5 and 1 µg/ml. The inhibition zone diameter of the extract and gentamicin extracts of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* were 8.33 and 7.45 mm. This research concludes that combination of extract *Aloe vera* and gentamicin sulphate can inhibit the growth of bacteria however activity was found to beequivalent to a single use.

Keywords: *Aloe vera* (L.) Burm.f. , gentamicin sulphate, FICI, *Pseudomonas aeruginosa, Staphylococcus epidermidis.*

INTRODUCTION

The most common treatment of infections is use antibiotic therapy.[1] However, the widespread use of antibiotics and improper use of the way will lead to new problems of bacterial resistance to antibiotics. An alternative way is to increase antibacterial effectiveness by combining extracts and antibiotics.

Aloe vera is a typical plant of West Kalimantan that has antibacterial activity. Extract ethanol of *Aloe vera* leaf contains terpenoids, steroids, tannins, saponins, anthraquinones[2], phenols and flavonoids.[3] According to research Tambekar (2007) states that fresh *Aloe vera* gel / sap can be a good antibacterial so infection caused by pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* can be cured.[4]

FICI values (Fractional Inhibitory Concentration Index) were used to evaluate the combination of two antibacterials in inhibiting bacterial growth. FICI values will show that the combination of test bacteria can be synergistic, antagonistic, additive, and no different.[5] FICI studies related to antibacterial combination were gallotannin 1,2,6-tri-O-galloyl-β-D-glucopyranose and gentamicin showed a synergistic effect on *Escherichia coli* bacteria with FICI value of 0.375.[6] *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* are common pathogenic aerobic bacteria causing infection.[7] Therefore, the present study aimed to determine the antibacterial activity and characteristics of combination of gentamicin sulphate and extract ethanol of *Aloe vera* leaf based on FICI values.

MATERIALS AND METHODS

The material used were leaf of *Aloe vera* from Jl. 28 October, North Pontianak, West Kalimantan. The chemicals used are gentamicin sulphate (Kimia Farma), Mueller Hinton Agar (Oxoid), aquadest, aqua pro injection, 0.9% NaCl solution, 96 % ethanol, 70 % ethanol, DMSO solution, and Mc Farland standard solution.

Aloe vera Leaves Extraction

The extraction process of *Aloe vera* leaves powder using maceration method. *Aloe vera* leaves powder weighed as much as 627.8 g and then soaked with 96% ethanol in a vessel with stirring until clear and solvent replacement every 24 hours for 7 days. The maseration results are filtered with white cloth and filter paper to separate the filtrate and residue. The filtrates were evaporated at a 50°C using rotary evaporator.

Bacteria Media and Inoculum Preparation

Dissolved 38 g of Mueller Hinton medium in a 1000 ml sterile distilled water, then heated and stirred until all dissolved, then the solution was sterilized using an autoclave of 121 ºC for 15 min.[8] Colonies of bacteria *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* taken from the work culture using sterile ose and then suspended into 10 ml of 0.9% sterile NaCl solution then incubated at 37ºC until obtained turbidity. Turbidity obtained then synchronized with Standard Mc. Farland 0.5 is equivalent to the growth rate of 1 x 10^8 bacterial cells / ml. If the turbidity is equal then the bacterial suspension can be used as a test bacteria.[9]

Minimum Inhibitory Concentration (MIC) Determination

MIC values were determined by using Kirby-Bauer diffusion method. Determination of MIC values include *Aloe vera* leaf extract, gentamicin sulphate and a combination of *Aloe vera* leaf extract and gentamicin sulphate. The concentration of extract solution for *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* were 10; 5; 2.5; 1.25; 0.625; and 0.3125 mg/mL while the concentration of gentamicin sulphate solution used were 8; 4; 2; 1; 0.5; and 0.25 µg/mL. Negative control and solvent extract used DMSO solution. The concentration of combined solution used were 1, ½ and ¼ of MIC value of ethanol extract of *Aloe vera* leaf and MIC gentamicin sulphate. The volume ratio used between the extract and gentamicin sulfate is 1: 1.

Fractional Inhibitory Concentration Index (FICI) Determination

FICI value (Fractional Inhibitory Concentration Index) of a combination of *Aloe vera* leaf skin extract and gentamicin sulphate was calculated based on the following formula:[10]

$$\sum FICI = FICI\ A + FICI\ B$$

$$\sum FICI = \frac{\text{MIC A in combination}}{\text{MIC A}} + \frac{\text{MIC B in combination}}{\text{MIC B}}$$

FICI values from a combination of *Aloe vera* leaf ethanol extract with gentamicin sulphate were analyzed descriptively.

RESULTS AND DISCUSSION

Minimum Inhibitory Concentration (MIC)

MIC value were determined using the Kirby-Bauer disc diffusion method. This method was used to see the sensitivity of antibacterial compounds. Principally the test compound will diffuse from the disc to the surface of the media. Parameters seen is whether there is a clear zone around the area of discs or not.[11]

MIC Ethanol extract of *Aloe vera* leaf

The ethanol extract of *Aloe vera* leaves tested antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* at concentrations of 10; 5; 2.5; 1.25; 0.625; and 0.3125 mg / ml. The solvent used to dissolve the extract is DMSO (Dimethyl Sulfoxide) because it has no antibacterial activity.[12] The results of this study do not show any inhibition zone. This is in accordance with previous research which states that DMSO is not able to inhibit bacterial activity.

Determination of MIC value show that *Aloe vera* leaf ethanol extract can inhibit the growth of *Pseudomonas aeruginosa* bacteria at concentration 10; 5 and 2.5 mg / ml with a succession zone of 12.02; 8.67 and 6.95 mm. At concentration 1.25 mg / ml did not show any inhibition zone so that MIC value of ethanol extract of *Aloe vera* leaf against *Pseudomonas aeruginosa* bacteria was found at concentration 2.5 mg / ml. This is in accordance with Kumar et al's study which states that the value of MIC extract of *Aloe vera* to *Pseudomonas aeruginosa* bacteria is 2.5 - 5 mg / ml.[13] Based on the observations presented in table 1 shows that *Aloe vera* leaf ethanol extract can inhibit the growth of *Staphylococcus epidermidis* bacteria at concentration 10; 5; 2.5; 1.25; and 0.625 mg / ml with a succession zone of 9.97; 8.95; 8.33; 7.92; and 7.47 mm. At concentration 0,3125 mg / ml did not show any inhibition zone so it can be concluded that MIC value is at concentration 0,625 mg / ml. The result of determination of MIC value of extract were tabulated in Table 1.

Ability of ethanol extract of *Aloe vera* leaf in inhibiting bacteria caused by active compound contained in it that is anthraquinone. There are several anthraquinone group compounds suspected to inhibit the growth of bacteria such as aloin A, aloin B, aloe emodin and isobarbaloin.[3] The mechanism of action of anthraquinone is to inhibit cell wall synthesis and bacterial nucleic acid synthesis. Anthraquinone will bind to the phosphate and insert itself on the base pair of DNA. This will affect the process of replication, transcription and expression of blocked genes will even lead to cell death. Other compounds also present in *Aloe vera* plants are phenols and flavonoids that can inhibit bacterial growth by destroying the permeability of bacterial cell membranes.[14]

MIC of Gentamicin Sulphate

Gentamicin sulphate were tested for inhibition of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* with concentration 8; 4; 2; 1; 0.5; and 0.25 µg / ml. Gentamicin sulphate was dissolved using aqua pro injection. Based on the inhibitory zone diameter table, the value of MIC gentamicin sulphate against *Pseudomonas aeruginosa* bacteria at concentration 8; 4; 2; 1; and 0.5 µg / ml can provide an inhibit zone each 15.58; 14.35; 11.53; 10.63; and 9.12 mm. Based on the inhibition zone results in table 2 it can be concluded that the MIC value of gentamicin sulphate is at a concentration of 0.5 µg / ml with a diameter of 9.12 mm, since the concentration of 0.25 µg / ml does not provide an inhibitory zone. Based on the inhibitory zone diameter table the value of MIC gentamicin sulphate against *Staphylococcus epidermidis* bacteria at concentration 8; 4; 2; 1; 0.5; and 0, 25 µg / ml can give an inhibit zone each 12.13; 10.35; 9.58; and 8.18 mm. Based on the inhibition zone results in table 2 it can be concluded that the MIC value of gentamicin sulphate is at a concentration of 1 µg / ml with a diameter of 8.18 mm because at a concentration of 0.5 µg / ml does not provide an inhibitory zone. The result of determination of MIC value of gentamicin sulphate were tabulated in Table 2.

Gentamicin sulphate is an aminoglycoside antibiotic. Gentamicin will enter the cell and bind strongly to the tRNA side component in the 16S RNA section of the 30S ribosomal subunit. The bond causes a mismatch between the codon and the anticodon resulting in an error in the translation. The incorrect translation of amino acids on the polypeptide chain will lead to an inappropriate type of protein.[15] Based on the table, the MIC gentamicin values obtained on testing of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* bacteria were respectively at concentrations of 0.5 µg / ml and 1 µg / ml. The results obtained were below the range of MIC values according to CLSI ranging from 4-16 µg / ml to *Pseudomonas aeruginosa* and *Staphylococcus* bacteria.[16] This indicates that the antibiotics used are still sensitive.

Fractional Inhibitory Concentration Index (FICI)

The combination solution was tested by Kirby-Bauer diffusion disc method with a volume ratio of 1: 1. The concentration used in combination to *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* bacteria. The result of determination of MIC value of combination were tabulated in Table 3.

The combination of ethanol extract of *Aloe vera* leaf and gentamicin sulphate leaves resulted in FICI value of 2, which means the combination characteristics are not different (indifferent) in both bacteria. This result show that the combination effect is no greater than the effect of ethanol extract of *Aloe vera* leaf and gentamicin from single used. The combination of *Aloe vera* leaf extract and gentamicin is expected to produce synergistic characteristics against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* bacteria.

The results of this study was different from the research hypothesis. The differences in the results of this study may be due to the extract used in this study still contains various compounds that may affect the inhibitory activity so fractionation or isolation efforts are required to know which compounds are responsible for synergistic activities.[17] In addition, there are metabolites of compounds produced by plants tested against bacteria that produce mechanisms that can affect the effectiveness of antibiotics.[18]

Based on the data from diameter of inhibition zone, combination obtained mean diameter to bacteria *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* respectively at 8.33 and 7.45 mm. This study has also been tested with ½ and ¼ concentrations of MIC values from ethanol extract of *Aloe vera* leaf and gentamicin sulphate with a volume ratio of 1: 1. However, there is no inhibition zone formed by the two bacteria. The diameter produced by the combination from ethanol extract of *Aloe vera* leaf and gentamicin sulphate on both bacteria did not increase compared to the diameter of ethanol extract of *Aloe vera* leaf and gentamicin in single used.

Table 1: Inhibitory zone diameter MIC ethanol extract of *Aloe vera* leaf skin

Concentration	Mean diameter of inhibition zone ($\bar{x}$± SD)	
	Pseudomonas aeruginosa	*Staphylococcus epidermidis*
10 mg/ml	12.02 ± 0.25	9.97 ± 0.32
5 mg/ml	8.67 ± 0.21	8.95 ± 0.31
2.5 mg/ml	6.95 ± 0.23	8.33 ± 0.35
1.25 mg/ml	0.00 ± 0.00	7.92 ± 0.33
0.625 mg/ml	0.00 ± 0.00	7.47 ± 0.32
0.3125 mg/ml	0.00 ± 0.00	0.00 ± 0.00
Negative control (DMSO)	0.00 ± 0.00	0.00 ± 0.00

Table 2: Inhibitory zone diameter MIC gentamicin sulphate

Concentration	Mean diameter of inhibition zone ($\bar{x}$± SD)	
	Pseudomonas aeruginosa	*Staphylococcus epidermidis*
8 µg/ml	15.58 ± 0.27	12.13 ± 0.27
4 µg/ml	14.35 ± 0.23	10.35 ± 0.22
2 µg/ml	11.53 ± 0.21	9.58 ± 0.26
1 µg/ml	10.63 ± 0.22	8.18 ± 0.27
0.5 µg/ml	9.12 ± 0.27	0.00 ± 0.00
0.25 µg/ml	0.00 ± 0.00	0.00 ± 0.00

Table 3: Inhibitory zone diameter MIC combination

Concentration extract + Gentamicin sulphate	Mean diameter of inhibition zone ($\bar{x}$± SD)
Pseudomonas aeruginosa	
2,5 mg/ml + 0,5 µg/ml	8.33± 0.20
1,25 mg/ml + 0,25 µg/ml	0.00 ± 0.00
0,625 mg/ml + 0,125 µg/ml	0.00 ± 0.00
Staphylococcus epidermidis	
0,625 mg/ml + 1 µg/ml	7.45 ± 0.28
0,3125 mg/ml + 0,5 µg/ml	0.00 ± 0.00
0,15625 mg/ml + 0,25 µg/ml	0.00 ± 0.00

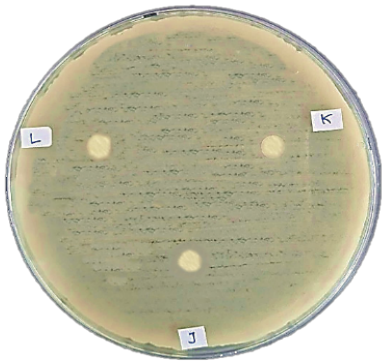

Figure 1: Combination ethanol extract of *Aloe vera* and gentamicin sulphate against *Pseudomonas aeruginosa*

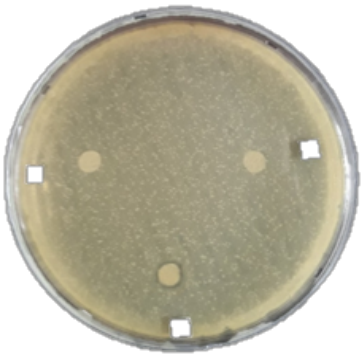

Figure 2: Combination ethanol extract of *Aloe vera* and gentamicin sulphate against *Staphylococcus epidermidis*

CONCLUSION

The combination of *Aloe vera* leaf extract (*Aloe vera* (L) Burm.f) and gentamicin sulphate can inhibit the growth of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* bacteria with inhibit zone respectively 8.33 ± 0.20 mm and 7.45 ± 0.28 mm. The combination of *Aloe vera* leaf extract (*Aloe vera* (L) Burm.f) and gentamicin sulphate have indifference effects on *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* bacteria with FICI values of 2.

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