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Determination of FICI Value of Combination of Ethanol Extract of *Aloe Vera* (L.) Burm. f. Leaf Peel and Chloramphenicol against Bacterial Pathogens

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ABSTRACT

Introduction: *The Increasing case of antibiotic resistance encourages a new action that is the combination of plant extracts and antibiotics. Aloe vera leaf skin (Aloe vera (L.) Burm f.), contains phenols, flavonoids and anthraquinones that act as antimicrobials.*

Objective: *The aim of research is to determine the value of FICI (Fractional Inhibitory Concentration Index) combination of ethanol extract of Aloe vera leaf skin (Aloe vera (L.) Burm f.), and chloramphenicol against pathogenic bacteria.*

Methods: *The determination of MIC (Minimum Inhibitory Concentration) value of ethanol extract for Aloe vera leaf skin (Aloe vera (L.) Burm. F.) and chloramphenicol use disc-paper diffusion method. The combination solution is created by a ratio of 1: 1 volumes from every MIC value. The determination of FICI values was analyzed by descriptive.*

Results: *The combination of Aloe vera leaf skin extract (Aloe vera (L.) Burm. F.) and chloramphenicol are used for Salmonella typhi bacteria which are 12.5mg / ml and 8 µg / ml, and for Bacillus subtilis bacteria is 2.5 mg / ml and 4 µg / ml resulted in inhibit zone 7.77 ± 0.25 and 7.83 ± 0.36. The combination of FICI value is 2 and it has indifference characteristics (not different) when the researchers compared between single extract and single chloramphenicol.*

Conclusion: *Aloe vera leaf skin ethanol extract (Aloe vera (L.) Burm. f.) and chloramphenicol may inhibit the growth of Salmonella typhi and Bacillus subtilis bacteria and both of the combinations show indifference characteristics of FICI 2 values.*

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INTRODUCTION

Infectious diseases are the main cause of high morbidity and mortality rates, especially in developing countries such as Indonesia (1). Treatment of infection generally uses antibiotic therapy. One of the efforts to overcome resistance and reduce side effects is to combine antibiotics with plants that have antibacterial activity.

Aloe vera plants contain secondary metabolites such as anthraquinones, tannins, saponins, terpenoids, steroids phenolics and flavonoids (2,3). Lawrence's research stated that the ethanol extract of aloe vera gel could inhibit the growth of *Salmonella typhi* and *Bacillus subtilis* bacteria (4). *Salmonella typhi* bacteria can cause typhoid fever, while *Bacillus subtilis* causes diarrhea if large numbers are found in the digestive tract.

FICI (Fractional Inhibitory Concentration Index) is used to interpret the results of a combination of two antibacterial compounds in inhibiting bacterial growth. The FICI value will indicate that the combination has synergistic, additive, indifference or antagonistic characteristics. This study aims to determine the effect and FICI value of the combination of the two compounds.

Based on this background, this study will use aloe vera plant extract which will be combined with antibiotics such as the one used in this study is chloramphenicol. This aims to overcome antibiotic resistance and drug side effects.

METHODS

Aloe vera Extract

Simplicia of aloe vera leaf skin is extracted by maceration method. The solvent used was 96% ethanol and solvent replacement was carried out every 24 hours for 7 days. Maserate was concentrated using a vacuum rotary evaporator at 500 ± C to obtain a thick extract (5).

Media Preparation

MHA media (Mueller Hinton Agar) weighed 38g, dissolved in 1 liter of distilled water while being heated and stirred with a magnetic stirrer. Then sterilized by autoclaving at 121ºC with a pressure of 15 psi (per square inch) for 15 minutes (6).

Bacterial Inoculums

Each colony of *Salmonella typhi* and *Bacillus subtilis* bacteria was taken from the rejuvenation culture overnight using a sterile loop needle and then suspended in 10 ml of 0.9% NaCl solution and then incubated at 37ºC until you get turbidity. The turbidity obtained was then adjusted to Standard Mc Farland 0.5, which is equivalent to the growth rate of 1 x 10^8 bacterial cells/mL (7).

Determination of Minimum Inhibitory Concentration (MIC) Value

The MIC value was determined by using aloe vera leaf extract and chloramphenicol as well as a combination of the two. The method used is paper disc diffusion. The concentration of aloe vera leaf skin extract against *Salmonella typhi* used was 25; 12.5; 6.25; and 3,125 mg/ml. As for B. subtilis 10; 5; 2.5; 1.25; 0.625; and 0.3125 mg/ml. The concentration of chloramphenicol used was 32; 16; 8; 4; 2; and 1 µg/ml. The solvent used was 20% DMSO and was used as a negative control. The volume ratio of the combined extract and chloramphenicol is 1 : 1. The MIC interpretation standard for chloramphenicol can be observed in Table 1.

Table 1. Criteria for Standard MIC Interpretation of Chloramphenicol against *Enterobacteriaceae* and *Bacillus*

Antibiotics	MIC Interpretation Criteria		
	Sensitive (µg/mL)	Intermediet (µg/mL)	Resistant (µg/mL)
Chloramphenicol	≤ 8	16	≥ 32

Each bacterial suspension was inoculated with a sterile loop needle on the surface of MHA media and placed on a 6 mm paper disc. Paper discs were dipped into the sample solution (extract solution, chloramphenicol solution, and combination solution) then placed on the surface of the MHA medium and pressed gently using sterile tweezers. After that the media was incubated in an incubator at 37°C for 18-24 hours and the diameter of the inhibition zone formed was measured (8). The categories of bacterial inhibition can be seen in Table 2.

Table 2. Category of Bacterial Inhibitory According to David Stout

Bacterial Inhibitory Power	Category
≥ 20 mm	Very Strong
10-20 mm	Strong
5-10 mm	Medium
≤ 5 mm	Weak

FICI Value Determination

The FICI value of the combination of aloe vera leaf peel extract and chloramphenicol was calculated based on the following formula (9):

$$\sum \text{FICI} = \text{FICI A} + \text{FICI B}$$

$$\sum \textbf{FICI} = \frac{\text{MIC A Combination}}{\textit{MIC A Single}} + \frac{\textit{MIC B Combination}}{\textit{MIC B Single}}$$

MIC A = aloe vera leaf peel extract
MIC B = chloramphenicol

The combination is considered to have a synergistic effects when the FICI value is ≤ 0.5. The combination has an additive effect when the value is 0.5 < FICI ≤ 1.0. The combination has an indifference effect when the value 1.0 < FICI ≤ 2.0. The combination has an antagonistic effect when the FICI value is > 2 (10).

Data analysis

The FICI value of the combination of ethanol extract of aloe vera leaf bark with chloramphenicol was analyzed descriptively.

RESULT

Extraction

The extracted dry powder was 2685.2 g. The weight of the extract after evaporation was 359.2g with a yield of 13.81%. Determination of Minimum Inhibitory Concentration (MIC) Value The inhibition zones of the ethanol extract of aloe vera leaf bark against *Salmonella typhi* and *Bacillus subtilis* can be seen in Table 3.

Table 3. MIC Inhibition Zone Diameter of Ethanol Extract of Aloe Vera Leaf Skin Against Pathogenic Bacteria

No.	Concentration (mg/ml)	Average Zone of Inhibition (± SD)*
Salmonella typhi		
1.	25	9.18 ± 0.28
2.	**12.5**	**7.46 ± 0.30**
3.	6.25	0.00 ± 0.00
4.	3.125	0.00 ± 0.00
Bacillus subtilis		
1.	10	8.55 ± 0.27
2.	5	7.35 ± 0.32
3.	**2.5**	**6.58 ± 0.25**
4.	1.25	0.00 ± 0.00
5.	0.625	0.00 ± 0.00
6.	0.3125	0.00 ± 0.00

*3 Replication

Based on the above table, the MIC values of the ethanol extract of aloe vera leaf bark against *Salmonella typhi* and *Bacillus subtilis* bacteria were 12.5 mg/ml and 2.5 mg/ml, respectively. Meanwhile, the diameter of the chloramphenicol inhibition zone for the two bacteria can be seen in Table 4.

Tabel 4. Results of Determination of MIC of Chloramphenicol Against Bacterial Pathogens

No.	Concentration (mg/ml)	Average Zone of Inhibition (± SD)*
Salmonella typhi		
1.	32	12.41 ± 0.38
2.	16	10.5 ± 0.32
3.	**8**	**8.06 ± 0.36**
4.	4	0.00 ± 000
5.	2	0.00 ± 0.00
6.	1	0.00 ± 0.00
Bacillus subtilis		
1.	32	12.75 ± 0.22
2.	16	10.28 ± 0.27
3.	8	8.30 ± 0.30
4.	**4**	**7.38 ± 0.35**
5.	2	0.00 ± 0.00
6.	1	0.00 ± 0.00

*3 Replication

Based on the table above, the MIC of chloramphenicol against *Salmonella typhi* and *Bacillus subtilis* bacteria was obtained, respectively, of 8 µg/ml and 4 µg/ml. The combination of extracts and antibiotics was carried out at 3 concentrations, namely 1, ½ , and ¼ times MIC , with a volume ratio of 1:1. The inhibition zone of the extract and chloramphenicol combination against *Salmonella typhi* and *Bacillus subtilis* bacteria can be seen in Table 5.

Tabel 5. Diameter of Inhibition Zone Combination of Ethanol Extract of Aloe Vera Leaf Peel and Chloramphenicol against Pathogenic Bacteria

MIC	Extract (mg/mL)	Chloramfenikol (µg/mL)	$\underline{x}$ ± SD
		Salmonella typhi	
1 x	**12.5**	**8**	**7.26 ± 0.28**
½ x	6.25	4	0.00 ± 0.00
¼ x	3.125	2	0.00 ± 0.00
		Bacillus subtilis	
1 x	**2.5**	**4**	**7.83 ± 0.36**
½ x	1.25	2	0.00 ± 0.00
¼ x	0.625	1	0.0 0.00

*3 Replication

FICI Value Determination

The MIC data of the ethanol extract of aloe vera leaf bark and chloramphenicol which had been obtained were used to determine the FICI value. The calculation of the FICI value can be seen in the formula below:

FICI Calculation on *Salmonella typhi* Bacteria

$$FICI = \frac{MIC\ Combination\ Extract}{MIC\ Single\ Extract} + \frac{MIC\ Combination\ Chloramphenicol}{MIC\ Single\ Chloramphenicol}$$

$$= \frac{12.5\ mg/mL}{12.5\ mg/mL} + \frac{8\ \mu g/mL}{8\ \mu g/mL}$$

$$= 1 + 1 = 2$$

FICI Calculation on *Bacillus subtilis* Bacteria

$$FICI = \frac{MIC\ Combination\ Extract}{MIC\ Single\ Extract} + \frac{MIC\ Combination\ Chloramphenicol}{MIC\ Single\ Chloramphenicol}$$

$$= \frac{2.5\ mg/mL}{2.5\ mg/mL} + \frac{4\ \mu g/mL}{4\ \mu g/mL}$$

$$= 1 + 1 = 2$$

DISCUSSION

MIC values were determined by the disc diffusion method. In this method, the test compound will diffuse into the agar. The observed parameter is the formation of an inhibition zone or a clear zone around the disc (11). The disc diffusion method is one of the methods recommended by CLSI in determining MIC.

The extract solvent and antibiotic or negative control used was Dimethyl-sulfoxide (DMSO). The results of this study showed no antibacterial activity and were in accordance with previous studies that DMSO had no antibacterial activity. Based on Table 3, the MIC value for the ethanol extract of aloe vera leaf bark against *Salmonella typhi* bacteria was 12.5 mg/mL. The results obtained from this study are in accordance with research conducted by Yebpella et al., (2011) which stated that the methanol extract of Aloe vera obtained from the National Research Institute for Chemical Technology (NARICT) in Zaria, has an MIC in the concentration range of 12.5-25 mg /mL against *Salmonella typhi* bacteria (12).

Based on Table 3, the MIC value for the ethanol extract of aloe vera leaf bark against *Bacillus subtilis* bacteria was 2.5 mg/mL. The results obtained from this study were not in

accordance with the research conducted by Abakar et al., (2017) which stated that the methanol extract of Aloe vera originating from Sudan had MIC ≤ 6.25 mg/mL against *Bacillus subtilis* bacteria (13).

The ability of the ethanol extract of aloe vera leaf bark to inhibit the growth of *Salmonella typhi* and *Bacillus subtilis* bacteria is caused by compounds in the extract that act as antibacterials. Compounds that are thought to have antibacterial activity are anthraquinones, phenols, and flavonoids. The mechanism of action of anthraquinones is the process of inhibiting bacterial nucleic acid synthesis which causes bacterial growth to be disrupted. Anthraquinone binds to nucleic acids and forms a complex that interferes with the function of the template DNA so that bacterial synthesis of DNA, RNA and protein is inhibited (14). Research by Pandey and Mishra, anthraquinone compounds in aloe vera are aloin, isobarbaloin, resistanol, and aloe emodin (15).

Other compounds that are thought to have antibacterial activity are phenols and flavonoids. Sharita et al., said that phenols and flavonoids have antibacterial activity by damaging the permeability of the bacterial cell wall (16). Phenol and flavonoid compounds will bind to proteins in bacteria through non-specific bonds to form protein-phenol complexes. At low concentrations, the complex will damage the cytoplasmic membrane and cause leakage of cell contents. At high concentrations, the complex will coagulate with cellular proteins and the cytoplasmic membrane undergoes lysis (17).

Based on Table 3, the MIC of the ethanol extract of aloe vera leaf bark against *Salmonella typhi* and *Bacillus subtilis* were 12.5 mg/mL and 2.5 mg/mL, respectively, with zones of inhibition of 7.46 mm ± 0.30 and 6.58 mm ± 0.25 which were in the moderate category.

The results of determining the MIC of chloramphenicol against *Salmonella typhi* and *Bacillus subtilis* bacteria can be observed in Table 4, indicating that chloramphenicol can inhibit the growth of *Salmonella typhi* bacteria at concentrations of 32, 16, and 8 µg/mL, with MIC values of 8 µg/mL and concentrations of 32, 16, 8, and 4 µg/mL for *Bacillus subtilis* bacteria, with an MIC value of 4 µg/mL.

Based on the data above, the MIC of chloramphenicol against Gram-negative bacteria is greater than that of Gram-positive bacteria. This happens because there are structural differences between the two bacteria. Where, Gram-negative bacteria have more layers of the cell wall than Gram-positive bacteria (18). Chloramphenicol is a broad spectrum antibiotic. Chloramphenicol acts by inhibiting protein synthesis. Chloramphenicol will bind to the 50s ribosome subunit and block the work of the ribosome, which interferes with the binding of new amino acids to the peptide chain that is being formed (19).

There are 2 important pathways by which antibiotics can penetrate the outer membrane of bacteria. The first is the lipid diffusion pathway for hydrophobic antibiotics and the porin diffusion pathway for hydrophilic antibiotics (20). Chloramphenicol is a hydrophobic antibiotic, because it does not dissolve in water (21). The structure of Gram negative bacteria, in this study, namely *Salmonella typhi*, consists of 3 layers, namely (1) outer membrane, which consists of lipopolysaccharides and phospholipids, (2) thin peptidoglycan, and (3) cytoplasmic membrane. Meanwhile, the structure of Gram-positive bacteria, namely *Bacillus subtilis*, consists of 2 layers, namely a thick peptidoglycan and a plasma membrane (22). Therefore, it takes a large concentration of chloramphenicol to penetrate the cell wall of Gram negative bacteria compared to Gram positive.

According to the CLSI on the interpretation standard MIC category table for Enterobacteriaceae and Bacillus, The MIC of chloramphenicol tested at concentrations of 8 and 4 µg/mL was included in the sensitive category in inhibiting *Salmonella typhi* and *Bacillus subtilis* bacteria. The MIC value is in accordance with the research of Padalia and Chanda (2015), which states that the MIC of chloramphenicol for *Bacillus subtilis* is 4 µg/mL and is in accordance with the research of Khanal et al., (2017), which states the MIC chloramphenicol against *Salmonella typhi* of 2 – 8 µg/mL (23,24).

FICI VALUE DETERMINATION

The FICI value is useful for interpreting the results of a combination of two antibacterial compounds in inhibiting bacterial growth. The MIC data of the ethanol extract of aloe vera leaf bark and chloramphenicol which had been obtained were used to determine the FICI value. Based on the above data, the average combined inhibition zone for *Salmonella typhi* and *Bacillus subtilis* with 1 x MIC was 7.26 mm and 7.83 mm, respectively. Comparison of ½ and ¼ MIC was also carried out but no inhibition zone was formed on both bacteria.

The combination of ethanol extract of aloe vera leaf peel and chloramphenicol against *Salmonella typhi* and *Bacillus subtilis* produced a FICI value of 2. This means that the characteristics of the combination are indifference (not different). Not different in meaning, the combined effect was not greater than the effect of single ethanol extract of aloe vera leaf bark and single chloramphenicol. This indifference characteristic occurs presumably because the two combined antibacterial compounds have the same mechanism of action in inhibiting bacteria. The ethanol extract of aloe vera leaf bark has an anthraquinone secondary metabolite which works by binding to nucleic acids and forming complexes that interfere with the function of template DNA so that protein synthesis is inhibited (3). Meanwhile, chloramphenicol will bind to the 50s ribosome subunit and interfere with protein synthesis (19). The similarity of this mechanism causes the two compounds to compete to be able to inhibit bacterial growth so that the effect that appears comes from only one compound.

The results obtained in this study were different from research conducted by Aelenei et al., that the combination of eugenol and chloramphenicol essential oils against *Salmonella typhi* bacteria had a synergistic effect with a FICI value of <0.5 (25). Olufunmi O et al., who stated that the combination of methanol extract of *Acacia mearnsii* (acacia) with chloramphenicol against *Bacillus subtilis* bacteria has synergistic characteristics with FICI 0.14 (26). However, according to research by Farooqui et. al which

stated that the combination of methanol extract of *Camellia sinensis* (tea) with chloramphenicol against *Salmonella typhi* bacteria had an indifference effect with a FICI value of 1.5 (27). The different FICI values in each study can be caused by differences in the content of secondary metabolites which result in different characteristics when combined.

CONCLUSION

The combination of ethanol extract of aloe vera leaf peel and chloramphenicol against *Salmonella typhi* and *Bacillus subtilis* produced inhibition zones of respectively 7.26 ± 0.28 and 7.83 ± 0.36. The FICI value of the combination of ethanol extract of aloe vera leaf peel and chloramphenicol is 2, which means it has indifference characteristics.

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