The Resistance Profile of Bacteria *Salmonella Sp* Towards Antibacterial Ethanol Extract of Bidara Leaves (*Ziziphus Mauritiana*)

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Keywords: Typhus, *Salmonella sp*,bacteria resistance, ethanol extract of leaves bidara (*Ziziphus mauritiana*), dilution.

Abstract: Typhus is an infection of the digestive tract which remains one of the health problems in Indonesia. Sanitation is a cause of the spread of the infection of *Salmonella sp.* Parts of plants such as leaves, stems, and roots are usually used as sources of treatment, especially for treating the typhus without prescription. Some Indonesian people consume the water of bidara leaves (*Ziziphus mauritiana*) to treat the disease. It can be explained by previous studies that the bidara leaves contains tannins and alkaloids which can prevent the growth of bacteria. The purpose of this experiment is to determine the resistance of *Salmonella sp* towards antibacterial ethanol extract of bidara leaves. The bacteria *Salmonella sp* was isolated from the blood sample of typhus patients on the extract in the Laboratory of Bacteriology. Antibacterial ethanol extract of bidara leaves had been tested with liquid dilution method consisted of six concentrations, namely 30%, 25%, 20%, 15%, 10% and 5%. The result of the MBC value from five *Salmonella sp* obtained from five different patients showed a significantly different. The extract of bidara leaves on sample 1, 2, 5 have the MBC value 15%; whereas in samples 3 and 4 have higher bactericides, namely at a concentration of 10%.

1 INTRODUCTION

Typhoid fever or known as typhus is the infection on the digestive tract. Infection on the alimentary canal caused by *Salmonella sp* specifically *Salmonella typhi* is remain existed as a common health problem among Indonesian people (Paputungan, 2016).

Moreover, in the case of typhus, Indonesia is in the third rank and WHO reported that the number of the sufferers increase each year in the approximate 800 per 100.000 people. This infection-related disease is spread widely in all Indonesian areas with the similar number of cases in each area (Yuswananda, 2015).

Previous studies showed that children aged between 6-12 (Umah, 2014) or between 3-19 (Nelwan, 2012) are at the highest risk for the potential of the typhus. Currently, this condition is worsened by the resistance of the bacteria *Salmonella sp*. Earlier in 1981, Sippel, et. al., reported that *Salmonella sp* resists to chloramphenicol (Hammad, et. al., 2011). The resistance appeared due to the use of unproperly anticiotic, for example, the take of a heavy dose of antibiotic (Febiana, 2012).

Bacterial resistant on the patients can worsen their poor health condition because it indicates that they need more doses of antibiotic drugs and the longer period is have to be taken in consuming the antibiotic. Meanwhile, the long-term of antibiotic consumption will finally defect the digestive system (Utami and Puspaningtyas, 2013).

The report of *Salmonella sp* resistance towards the chemical antibiotic became the baseline for us to find new antibacterial; another viable alternative from plants as an antibiotic resource without negative side effects on the human body especially on the digestive canal. Plants as natural resources can be taken as either prevention or treatment because plants contain many active compounds which function as antibacterial, antioxidant, anti-inflammation and immunomodulator (Karon, et. al., 2011). Further, work by Karon, et. al., (2011) proves

Mukhlishoh, F., Verdianti, P. and Krihariyani, D.
The Resistance Profile Of Bacteria Salmonella Sp Towards Antibacterial Ethanol Extract Of Bidara Leaves (Ziziphus Mauritiana).
DOI: 10.5220/0007543403810385
In *Proceedings of the 2nd International Conference Postgraduate School (ICPS 2018)*, pages 381-385
ISBN: 978-989-758-348-3

that ethanol extract of Bidara leaves (*Ziziphus mauritiana*) demonstrates a positive response in the prevention of the growth of some bacterias: *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus*, and *Vibrio cholera*.

In addition, the Bidara leaves are utilized empirically by group of people in Lenteng village, Sumenep Regency, to treat common fever and typhus. However, the consumption of the leaves is regardless of the scientific knowledge about the active compounds contain inside the leaves; as a consequence, they traditionally sublime the leaves without knowing the proper composition. Ironically, Bidara as wild plants were cropped so that recently it is difficult to find Bidara trees around people's house even for some people who are taking the leaves to treat the family member who is suffering from common fever or typhus.

Because of the resistance of bacteria of *Salmonella sp* in antibiotics has been reported and also people haven't known the benefits of plants such as bidara leaves, thus this research is a continuity of the research that has been done by Karon et al. (2011). The research by Karon et al., was about the antibacterial resistance of ethanol extract of bidara leaves (*Ziziphus mauritiana*) against *Salmonella typhi* bacteria and *Salmonella paratyphi* ATCC. Therefore, this research will use *Salmonella sp* bacterias which isolated from the blood of typhoid fever patients because ATCC bacteria may not be able to describe the bacteria that attack the community so that the resistance profile of the *Salmonella sp* bacteria in the blood of typhoid fever patients in that extract will be known.

2 METHOD

Post-test through experimental design was used to examine the antibacterial power of Bidara leaves' ethanol extract towards *Salmonella sp* bacteria by using liquid dilution method. We took *Salmonella sp* bacteria which was isolated from the blood sample of typhus patients. It is done because the typhus caused by ATCC bacteria had not been concluded yet that it is which infected the patients so that the resistance profile of *Salmonella sp* bacteria on the typhus patients' blood to the antibacterial compound of the extract would be known.

2.1 Extraction Substance

The Bidara leaves were gotten from Lenteng Village which is located in Sumenep Regency, Madura. Firstly, the leaves were dried and sublimated until it obtained 250 gram of the leaf powder. Secondly, the powder was extracted by using ethanol solvent 95 % through the maceration method; the maceration process was running within 5 days by changing the solvent every day. Thirdly, filtrate was collected every 24 hours of immersion in which each filtrate was mixed and evaporated by rotary evaporator at 40° C and 120 rpm of speed so that we gained 100 % of ethanol extract of Bidara leaves.

The used of concentration were 30 %, 25 %, 20 %, 15 %, 10 %, and 5 %. Following were the method of obtaining the extract through each concentration:

2.1.1 Concentration 30 % : 30 µl bidara leaves extract 100 % + 70 µl DMSO
2.2.1 Concentration 25 % : 25 µl bidara leaves extract 100 % + 75 µl DMSO
2.3.1 Concentration 20 % : 20 µl bidara leaves extract 100 % + 80 µl DMSO
2.4.1 Concentration 15 % : 15 µl ml bidara leaves extract 100 % + 85 µl DMSO
2.5.1 Concentration 10 % : 10 µl bidara leaves extract 100 % + 90 µl DMSO
2.6.1 Concentration 5% : 5 µl bidara leaves extract 100 % + 95 µl DMSO.

Ciprofloxacin antibiotic was used for the positive control with concentration 30 %, 25 %, 20 %, 15 %, 10 %, and 5 %. These were the method to modulate the expected concentration of ciprofloxacin:

2.1.1 Concentration 30 % : 0,03 gram tablet of ciprofloxacin + 100 µl sterile aquades
2.2.1 Concentration 25 % : 0,025 gram tablet of ciprofloxacin + 100 µl sterile aquades
2.3.1 Concentration 20 % : 0,02 gram tablet of ciprofloxacin + 100 µl sterile aquades
2.4.1 Concentration 15 % : 0,015 gram tablet of ciprofloxacin + 100 µl sterile aquades
2.5.1 Concentration 10 % : 0,01 gram tablet of ciprofloxacin + 100 µl sterile aquades
2.6.1 Concentration 5 % : 0,005 gram tablet of ciprofloxacin + 100 µl sterile aquades

2.2 Bacterial Suspension Preparation

In this research, there were two kinds of bacterial suspensions that been used. One was *Salmonella sp* which was isolated from the blood sample of typhus patients placed as the experimental group. This suspension consisted of 5 suspensions in which the bacterias were collected from 5 different patients. And the other one was bacteria suspension of *Salmonella sp* ATCC which was placed as a positive and negative control. The bacterial suspension was

made by mixing the bacteria colony that grew on the media *Nutrient Agar Slant* (NAS) into sterile 2 ml PZ, then the turbidity was equated to the standard turbidity of McFarland 0,5.

2.3 Liquid Dilutin Method

Each extract solvent with concentration 30 %, 25 %, 20 %, 15 %, 10 %, and 5 % was inoculated with 5 bacteria *Salmonella sp* suspensions from the blood sample of patients, where each blood sample was added one loop of bacteria suspension. The similar procedure was also implemented to the positive control with the *Salmonella sp* ATCC bacteria suspension for each ciprofloxacin solvent. In different from that, for the negative control was used sterile aquades to be inoculated with one loop of *Salmonella sp* ATCC bacteria suspension.

After completing the inoculation with bacterial suspension, then each was incubated into an incubator at 37 °C during 24 hours. After 24 hours, it was observed to know the presence or the absence of the turbidity in each tested solvent. Continually, the subculture of tested-mixed solvent was undertaken to conclude whether the bacteria grew or not by using bacteria suspension which had incubated on the media Mueller Hinton Agar (MHA) by a strict method. The incubation was arranged at 37 °C for 24 hours.

The Minimum Bactericidal Concentration (MBC) was determined from the lowest concentration which can reduce the viability of the bacteria; whereas the Minimum Inhibitory Concentration (MIC) was determined by the lowest level of the bacterial agent that inhibits the growth (Vineethe, et. al, 2015).

2.4 Data Analysis

The data were analyzed by Kruskal Wallis statistical method to know the difference of resistance profile on *Salmonella sp* bacteria in the blood of typhus patients towards the antibacterial of *Ziziphus mauritiana* leaves' ethanol extract.

3 FINDINGS AND DISCUSSION

These are the laboratory test results of antibacterial ethanol extract of Bidara leaves towards *Salmonella sp* bacteria compared to positive control and negative control.

Tabel 1: Laboratory test result of antibacterial extract of *Ziziphus mauritiana* leaves on *Salmonella sp* in the patients' blood sample using liquid dilution method

C	R	1		2		3		4		5		Ct (+)		Ct (-)	
		T	S	T	S	T	S	T	S	T	S	T	S	T	S
30%	R_1	-	-	-	-	-	-	-	-	-	-	-	-		
	R_2	-	-	-	-	-	-	-	-	-	-	-	-		
	R_3	-	-	-	-	-	-	-	-	-	-	-	-		
	R_4	-	-	-	-	-	-	-	-	-	-	-	-		
25%	R_1	-	-	-	-	-	-	-	-	-	-	-	-		
	R_2	-	-	-	-	-	-	-	-	-	-	-	-		
	R_3	-	-	-	-	-	-	-	-	-	-	-	-		
	R_4	-	-	-	-	-	-	-	-	-	-	-	-		
20%	R_1	-	-	-	-	-	-	-	-	-	-	-	-		
	R_2	-	-	-	-	-	-	-	-	-	-	-	-		
	R_3	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	R_4	-	-	-	-	-	-	-	-	-	-	-	-		
15%	R_1	-	-	-	-	-	-	-	-	-	-	-	-		
	R_2	-	-	-	-	-	-	-	-	-	-	-	-		
	R_3	-	-	-	-	-	-	-	-	-	-	-	-		
	R_4	-	-	-	-	-	-	-	-	-	-	-	-		
10%	R_1	+	+	+	+	-	-	-	-	+	+	-	-		
	R_2	+	+	+	+	-	-	-	-	+	+	-	-		
	R_3	+	+	+	+	-	-	-	-	+	+	-	-		
	R_4	+	+	+	+	-	-	-	-	+	+	-	-		
5%	R_1	+	+	+	+	+	+	+	+	+	+	-	-		
	R_2	+	+	+	+	+	+	+	+	+	+	-	-		

R_3	+	+	+	+	+	+	+	+	+	+	-	-
R_4	+	+	+	+	+	+	+	+	+	+	-	-
MBC	15%		15%		10%		10%		15%		5%	
MIC	10%		10%		5%		5%		10%			

Table Information:
C = Concentration
T = Turbidity
+ : Turbid
- : Unturbid
S = Sublimation on the media MHA
+ : bacteria are grown
- : bacteria are not grown

The test group 1, 2, 3, 4, 5 were using *Salmonella sp* bacteria which isolated from the patients' blood sample and bidara leaves' ethanol extract by using each concentration
Ct (+) = Positive control contains ciprofloxacin with concentrations which were inoculated by the bacteria *Salmonella sp* ATCC
Ct (-) = Negative control contains sterile aquades that was inoculated by the bacteria *Salmonella sp* ATCC
MBC = the Minimum Bactericidal Concentration
MIC = the Minimum Inhibitory Concentration

3.1 Statistical Test Result

Table 2: The result of Kruskal Wallis statistical method

	Turbidity of testing sorvent	The growth of bacteria *Salmonella sp*
Chi-Square	36,000	36,000
df	36	36
Asymp. Sig.	,046	,046

The data on the table are interpreted by these guidelines:
If the significance value (P) ≥ α, then H_0 is accepted and H_1 is rejected
If the significance value (P) < α, then H_0 is rejected and H_1 is accepted

The table shows that p-value is 0.046 and α is 0.05. In comparison p < α which means H_0 is rejected and H_1 is accepted. Hence, there is a difference on the resistance profile of bacteria *Salmonella sp* to Bidara leaves' ethanol extract.

3.2 Discussion

There are different findings resulted from blood sample of typhus patients where some concentrations used are 30 %, 25 %, 20 %, 15 %, 10 % and 5 %. On sample 1, 2, and 5 the bacteria could be grown at concentration 5 % and 10 %; meanwhile, on sample 3 and 4, the bacteria could be grown at concentration 5 %.

Bidara leaves contain tannin and alkaloid compounds as antibacterial to inhibit and even kill bacterial growth. The mechanism of inhibition and killing of bacterial growth by tannins and alkaloids that contained in Bidara leaves extract occurs when the extract solution is inoculated with bacteria and then incubated at 37° C for 24 hours.

The growth of bacteria at certain concentrations happened due to the fact that other compounds such as tannin an alkaloid contained in the ethanol extract of Bidara leaves could not kill the bacteria; as a result, the bacteria had become grown. In addition, at concentrations where the viability of the bacteria was not found show that tannin and alkaloid could kill the bacteria *Salmonella sp*.

Tannin and alkaloid that contained in the extract of Bidara leaves work together in killing the bacteria by protein denaturation and inhibit the nucleic acid bacteria. At first, the alkaline cluster of both of the active compounds which contain nitrogen will react with the DNA bacteria, which is the main component of nucleus. By that process, the protein synthetic and nucleic acid within cells will be disrupted so that the metabolism of the bacteria cells will also be disrupted and the bacteria growth will be inhibited and even killed (Roslizawaty, et. al., 2013).

Moreover, tannin will also disrupt the wall of the bacterial cells by creating polysaccharide complex which can cause the permeability of the bacteria cells disturbed. The disturbing of bacteria cells permeability cause the cells cannot be active; consequently, the growth of bacteria will be inhibited and also killed (Ustiawaty, et. al., 2015).

The positive control by using ciprofloxacin at concentration 30 %, 25 %, 20 %, 15 %, 10 %, and 5 % demonstrated that there was no bacteria growth. It's because ciprofloxacin has the ability to prevent the forming of gyrase which is the cause of opening and formation of the superhelix on the DNA

bacteria. When the forming enzyme is inhibited, thus the bacteria cannot be replicated.

Different from the positive control, the negative control cannot kill the bacteria so that they can grow because the sterile aquades does not contain any antibacterial compound to kill the bacteria.

Based on the sample, it is known that there is no genetic mutation or resistance on the bacteria. This conclusion refers to the difference of MBC value from each sample. The value of positive control is 5 %, the MBC of sample 1, 2, and 5 at concentration 15 %, and the MBC of sample 3 and 4 are 10 %. The other value that also can be understood is that the ethanol extract of Bidara leaves is unsignificant in treating the infection caused by *Salmonella sp*.

4 CONCLUSIONS

Ethanol extract of Bidara leaves can kill the growth of bacteria *Salmonella sp* at concentration 30 %, 25 %, 20 %, and 15 %. This finding explicated that the extract cannot be taken as an alternative treatment because the bactericidal concentration is under ciprofloxacin. The MBC sample 1, 2, and 5 are 15 % while the sample 3 and 4 are 10 %.

The difference of the MBC values indicated that the profile of *Salmonella sp* bacteria is different on the Bidara leaves' ethanol extract. Furthermore, from the MBC values, it can be concluded that there are no bacteria which is resistant or changed genetically.

ACKNOWLEDGEMENTS

The acknowledgment is conveyed to my academic mentors who were supervising me in the whole process of research and in writing the results findings. Both of my mentors, Dwi Krihariyani and Nur Cholis Al Anwary, are my undergraduate thesis supervisor at the Department of Analis Kesehatan, Poltekkes Kemenkes Surabaya.

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