The Effect of Cocoa Leaf Extract (*Dendrophthoe pentandra* (L.) Miq.) on The Growth of *Shigella dysenteriae* and *Salmonella typhi*

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Abstract

Cocoa parasite leaves (Dendrophthoe pentandra (L.) Miq) belong to parasitic plants against hosts which are potentially used as traditional medicinal ingredients. However, the efficacy of cocoa parasite leaves is still not widely known by the public. One of the properties from cacao parasitic leaves is able to treat mild diseases such as smallpox, hookworm, flu, cough and diarrhea as well as being able to be used as a treatment for serious diseases such as tumors and cancer. The bacteria causing infection generally come from gram negative bacteria, namely Shigella dysenteriae and Salmonella typhi which refer to enteropathogenic bacteria causing infection to the host. The existing controls still use antibiotic drugs that are used less procedurally and causing bacteria more resistant. Cocoa parasite leaf extract contains active compounds in the form of alkaloids and saponins which play a role in inhibiting bacterial growth. This study aims to determine the effective concentration on the effect of cocoa parasite leaf extract to the growth of Shigella dysenteriae and Salmonella typhi bacteria. The research method was by using diffusion so that by using wells with 5 repetitions. The concentration used was 8%, 10%, 12%, 14%, 16%. Data were analyzed by using ANOVA analysis to determine the effective concentration on the effect of extracts to the inhibition of bacterial growth. The significant values were indicated at 0,000 against Shigella dysenteriae and 0.042 bacteria against Salmonella typhi (<0,05). The data revealed that cocoa parasite leaf extract has the potential to inhibit the growth of Shigella dysenteriae and Salmonella typhi bacteria.

Keywords: *Cocoa Leaf Extract, Shigella dysenteriae, Salmonella typhi*

1. INTRODUCTION

Cocoa *parasite leaves* (*Dendrophthoe pentandra* (L.) Miq) is Diarrhea is a set of symptoms characterized by bowel movements with the watery stools. Diarrhea is categorized parasitic plant that can be used as antimicrobials[1]. This misleto is known to cure diseases such as smallpox, hookworm, flu, cough, diarrhea, tumor and cancer[2]. into two, namely infectious and non infectious diarrhea. Infectious diarrhea is caused by microorganisms, such as bacteria, viruses and parasites. Meanwhile, non-infectious diarrhea can be caused by psychological factors due to anxiety or fear[3]. This disease frequently spreads in public and commonly medications are prescribed for the patients. However, the medicine is often overused and it causes side effects to the body[4] and the resistance of bacteria. Additionally, this type of Indonesian *parasite leaves*s (*Dendrophthoe pentandra* (L.) Miq) contains active compounds such as alkaloids and saponins[5].

Alkaloid compounds work by inhibiting the components of bacteria cell peptidoglycan, so that the bacteria cell layer is not fully formed and die[6].

Saponin works as an antibacterial by disrupting the stability of bacteria cell membranes, causing bacteria cells to be lysis. The mechanism of saponins in the antibacterial group is disrupting the permeability of bacteria cell membranes which results damage to it and causes the release of various important components in bacteria cells including proteins, nucleic acids and nucleotides. Saponin is also able to inhibit bacteria growth by limiting the mechanisms for protein synthesis and causing changes in bacteria cells[7].

Shigella dysenteriae and *Salmonella typhi* bacteria are enteropathogenic bacteria that cause infection. *Shigella dysenteriae* is

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facultative anaerobes living in the human digestive tract which can cause bacillary dysentery. Whereas, *Salmonella typhi* is gram-negative bacteria that belong to facultative anaerobes. Its habitat is in human's and animal's digestion. The ability of *Salmonella typhi* to invade and damage cells is related to the production of thermostable cytotoxic factor, it directly affects the secretion of water and electrolytes[8].

Ethanol extract and ethyl acetate of *parasite leaves* have been tested on the bacteria *Klebsiella pneumonia*. From the results of these tests, it was indicated that the presence of antibacterial potential in inhibiting the growth of *Klebsiella pneumonia* bacteria which is stated by an effective concentration of 50%[9]. The minimum inhibitory concentration test of cocoa *parasite leaves* (*Dendrophthoe pentandra* (L.) Miq) has never been carried out on enteropathogenic bacteria such as *Shigella dysenteriae* and *Salmonella typhi*. Based on the explanation above, it is necessary to conduct a research on "The Effect of Cocoa *Parasite leaves* Extract (*Dendrophthoe pentandra* (L.) Miq) on *Shigella dysenteriae* and *Salmonella typhi* Bacterial Growth".

2. RESEARCH METHOD

The method used in this research is laboratorial experiment using well diffusion method. The researcher spread the cocoa *parasite leaves* extract into the plate so that a zone of inhibition occurred in the *Shigella dysenteries* and *Salmonella typhus* bacterial colonies. The treatment of cocoa *parasite leaves* extracts (*Dendrophthoe pentandra* (L.) Miq) consisted of 5 concentrations, namely (8%, 10%, 12%, 14%, 16%). Each treatment consisted of five repetitions.

Place and Time of Research

This research was carried out at Microbiology Laboratory of Biology Education Study Program, the University of Jember, Faculty of Teacher Training and Education and at Jenggawah Research Center for sampling the cocoa *parasite leaves* (*Dendrophthoe pentandra* (L.) Miq). The study was conducted from December to March 2019.

Tools and Materials

The material used in this study was cocoa *parasite leaves* (*Dendrophthoe pentandra* (L.) Miq), cultivation of *Shigella dysenteriae* and *Salmonella typhi* bacteria obtained from the Laboratory of Microbiology, Medical Faculty of Jember University, Nutrient Agar (solid media), Nutrient Broth (liquid media), sterile distilled water as negative control and ciprofloxacin as positive control, 70% ethanol solution, 70% alcohol, crystal violet, lugol solution, safranin, aluminum foil, sterile cotton.

Data Analysis

In determining the effective concentration of the effect of cocoa *parasite leaves* (*Dendrophthoe pentandra* (L.) Miq) on the growth of *Shigella dysenteriae* and *Salmonella typhi* bacteria, a minimum inhibitory concentration test was carried out. Then, the research results were analyzed by using ANOVA to determine the effect of effective concentration of extracts on the growth of *Shigella dysenteriae* and *Salmonella typhi* bacteria. The results of the analysis were then followed by LSD testing to find out that each serial extract concentration given was significantly different.

3. RESULTS AND DISCUSSION

Results

From the analysis, it can be concluded that the results of the effective concentration test can inhibit the *Shigella dysenteriae* and *Salmonella typhi* bacteria with serial concentrations of 8%, 10%, 12%, 14%, 16%. The test results of cocoa *parasite leaves* (*Dendrophthoe pentandra* (L.) Miq), *Shigella dysenteriae*he, and *Salmonella typhi* bacteria can be seen in Table 1 and Table 2.

Table 1. The results of the effective concentration test of cocoa *parasite leaves*extract (*Dendrophthoe pentandra* (L.) Miq) against *Shigella dysenteriae* bacteria.

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Treatment	Inhibited Zone Average (mm)	Standart deviasi
P1	1,38	± 0,32461
P2	1,74	± 0,22163
P3	2,04	± 0,31525
P4	1,62	± 0,08307
P5	2,94	± 0,31302

Descriptions:

P1: 8% cocoa parasite leaf extract

P2: 10% cocoa parasite leaf extract

P3: 12% cocoa parasite leaf extract

P4: Cocoa parasite leaf extract 14%

P5: Cocoa parasite leaf extract 16%

From Table 1, it shows that at a concentration of 8% there are obstacles with an average resistance of 1.38 mm so the greater the concentration of extract, the bigger inhibitory zone was formed, it was proved by the concentration of 16% with an average inhibition zone of 2.94 mm. As for the results of the test the effective concentration of cocoa *parasite leaves* against *Salmonella typhi* bacteria can be seen in Table 2 below.

Table 2. The results of the effective concentration test of cocoa *parasite leaves* extract (*Dendrophthoe pentandra* (L.) Miq) against *Salmonella typhi* bacteria.

Treatment	Inhibited Zone Average (mm)	Standart deviasi
P1	1,36	± 0,30992
P2	2,1	± 0,41100
P3	2,48	± 0,34717
P4	3,62	± 0,29925
P5	4,6	± 0,54420

From table 2, it can be concluded that at a concentration of 8% an inhibition zone has been formed with an average resistance of 1.36 mm so, the greater the concentration of extracts, the wider inhibitory zone was formed. It was proved by the concentration of 16% with a mean inhibition zone of 4.6 mm. The influence of the inhibitory zone of the cocoa *parasite leaves* extract (*Dendrophthoe pentandra* (L.) Miq) on the *Shigella dysenteriae* and *Salmonella typhi* bacteria can be seen in Figure 1.

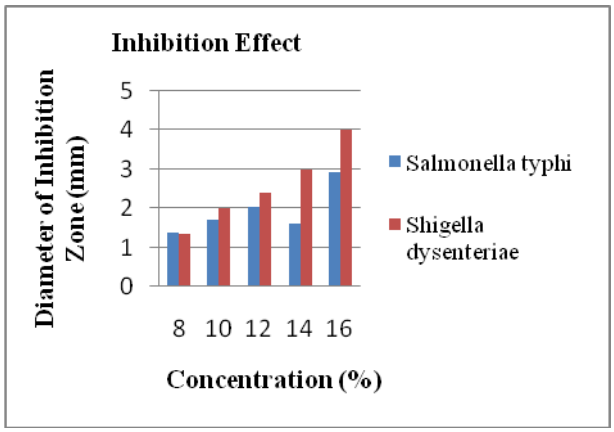

Figure 1. Graph of the effect of cocoa *parasite leaves* extract (*Dendrophthoe pentandra* (L.) Miq) on *Shigella dysenteriae* and *Salmonella typhi* bacteria

Effective concentration test analysis using One Way ANOVA test with a significant level of 95% (<0.05). The analysis of the effective concentration of *Shigella dysenteriae* and *Salmonella typhi* bacteria can be seen in Table 3 and Table 4.

Table 3. The results of ANOVA test analysis on the effective concentration of *Shigella dysenteriae* bacteria extract of cocoa leaves (*Dendrophthoe pentandra*(L.) miq).

ANOVA

Obstacles zone

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2,788	4	,697	9,714	,000
Within Groups	1,435	20	,072		
Total	4,223	24			

Table 4. The results of ANOVA test analysis on the effective concentration of *Salmonella typhi* bacteria extract of cocoa leaves (*Dendrophthoe pentandra*(L.) miq).

ANOVA

Obstacles zone

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,862	4	,465	3,018	,042
Within Groups	3,085	20	,154		
Total	4,947	24			

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The results of further LSD testing in *Shigella dysenteriae* and *Salmonella typhi* bacteria can be seen in Tables 5 and 6.

Table 5. The results of LSD test analysis on *Shigella dysenteriae* bacteria.

Dependent Variable: Zonahambat

	(I) Konsentrasi	(J) Konsentrasi	Mean Difference (I-J)	Std. Error
LSD	8%	10%	,13600	,16941
		12%	,76400*	,16941
		14%	,68800*	,16941
		16%	,77200*	,16941
	10%	8%	-,13600	,16941
		12%	,62800*	,16941
		14%	,55200*	,16941
		16%	,63600*	,16941
	12%	8%	-,76400*	,16941
		10%	-,62800*	,16941
		14%	-,07600	,16941
		16%	,00800	,16941
	14%	8%	-,68800*	,16941
		10%	-,55200*	,16941
		12%	,07600	,16941
		16%	,08400	,16941
	16%	8%	-,77200*	,16941
		10%	-,63600*	,16941
		12%	-,00800	,16941
		14%	-,08400	,16941

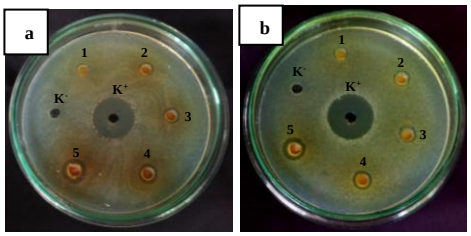
*. The mean difference is significant at the 0.01 level.

Table 6. The results of LSD test analysis on *Salmonella typhi* bacteria.

Dependent Variable: Zona hambat

	(I) Konsentrasi	(J) Konsentrasi	Mean Difference (I-J)	Std. Error
LSD	8%	10%	,05200	,24839
		12%	,07400	,24839
		14%	-,03000	,24839
		16%	,70000	,24839
	10%	8%	-,05200	,24839
		12%	,02200	,24839
		14%	-,08200	,24839
		16%	,64800	,24839
	12%	8%	-,07400	,24839
		10%	-,02200	,24839
		14%	-,10400	,24839
		16%	,62600	,24839
	14%	8%	,03000	,24839
		10%	,08200	,24839
		12%	,10400	,24839
		16%	,73000*	,24839
	16%	8%	-,70000	,24839
		10%	-,64800	,24839
		12%	-,62600	,24839
		14%	-,73000*	,24839

The results of ANOVA analysis in Tables 3 and 4, on *Shigella dysenteriae* and *Salmonella typhi* bacteria revealed that the treatment using cocoa parasite leaf extract on the growth of *Shigella dysenteriae* and *Salmonella typhi* bacteria with each serial concentration of 8%, 10%, 12%, 14 %, 16% showed a significant value of 0,000 in *Shigella dysenteriae* bacteria and 0,042 in *Salmonella typhi* bacteria. From the results of the final test analysis it can be concluded that the data analysis results are significant because the results explained a number of <0.05, which means that there is a significant effect of serial cocoa parasite leaf extract concentration (*Dendrophthoe pentandra* (L.) miq) on *Shigella dysenteriae* and *Salmonella typhi* bacterial growth. The LSD test results revealed that the effective concentration of *Shigella dysenteriae* was shown at a dose of 12% and 16% in *Salmonella typhi*, which means that there was a significant or significantly different effect because the data from the analysis showed (<0.05). The final test results for the influence of cocoa parasite leaf extract (*Dendrophthoe pentandra* (L.) miq) on the *Shigella dysenteriae* and *Salmonella typhi* bacteria growth can be seen in Figure 2 below.



Note :
1 : 8% cocoa parasite leaf extract
2 : 10% cocoa parasite leaf extract
3 : 12% cocoa parasite leaf extract
4 : 14% cocoa parasite leaf extract
5 : 16% cocoa parasite leaf extract
K (+) : Ciprofloxacin 0,001%
K (-) : Aquades steril

Figure 2. (a) The final test results on the effect of cocoa parasite leaf extract (*Dendrophthoe pentandra* (L.) Miq) on *Shigella dysenteriae* bacteria. (b) The final test results on the effect of cacao parasite leaf extract (*Dendrophthoe pentandra* (L.) Miq) on *Salmonella typhi* bacteria.

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Discussion

Based on the results of the final test using a concentration of 8%, 10%, 12%, 14%, 16% from cocoa parasite leaf extract on the growth of *Shigella dysenteriae* bacteria, it revealed that at a concentration of 8% with a mean resistance of 1.38 mm showed the existence of obstacles. Whereas in *Salmonella typhi* bacteria, it showed the same concentration that was 8% with different inhibitory zone average that was equal to 1.36 mm indicating the existence of inhibition zone. From the results of further LSD tests, it showed that in Shigella bacteria dysenteriae the effective concentration was shown at a dose of 12% and 16% in *Salmonella typhi* which means that the data was significantly influential / significantly different. Based on these results it indicated that the cacao leaf parasite extract can inhibit the growth of *Shigella dysenteriae* and *Salmonella typhi* bacteria due to the presence of active compounds such as alkaloids and saponins functioning to damage bacterial cell walls. From the test results, it showed that the inhibitory power of the extract was still far below the positive control of ciprofloxacin. This is because the working ability of the active compound of the cocoa parasite leaves is not as fast as the ciprofloxacin antibiotic. Although the ability to work is not as fast as ciprofloxacin, but cocoa parasite leaf extract will not cause side effects on the body because the active compound formed is naturally by plants. While antibiotics (ciprofloxacin) will continuously cause the bacteria to become resistant and cause an impact on the body [4].

In vitro testing using the diffusion method to use the wells determined the resistance to the growth of *Shigella dysenteriae* and *Salmonella typhi* bacteria which can be identified by the presence of a barrier zone around the wells. The resulting inhibition zone has different diameters of the concentration extract. The smaller or lower the concentration of the extract, the less active compounds are contained in it. Therefore the inhibitory zone will also be smaller. This indicates that if the concentration of the material is higher, the resistance of microbes will be lower [10].

The effect of cocoa parasite leaf extract (*Dendrophthoepentandra* (L.) Miq) from both bacteria was due to differences in cell wall structure. The cell wall of *Shigelladysenteriae* bacteria, as gram negative, consists of a peptidoglycan layer which is easily penetrated by active compounds on the cell wall causing more susceptible to physical shocks, such as giving antibiotics or other antibacterial ingredients [11].

Antibacterial compounds are able to damage the peptidoglycan layer in *Shigella dysenteriae*, so that when the extract of cocoa parasite leaves being inoculated on the medium it will damage the cell wall layer. The inhibition zone formed around the well indicates that the bacteria are susceptible to the defense of the cell wall that has been damaged by the treatment of cocoa parasite leaf extract.

In *Salmonella typhi* it is also able to be inhibited, but the inhibition process is faster in *Shigella dysenteriae.*

This is because the *Salmonella typhi* bacteria have a higher level of resistance. With the formation of a complex bacterial cell wall, a complex lipopolysaccharide arrangement on the bacterial cell wall consisting of a lipopolysaccharide endotoxin arrangement on the cell wall, namely specific O polysaccharide which is a somatic antigen that induces specific immunity, intipolisaccharide which induces some specific resistance to gram negative sepsis , lipid A with KDO (2-keto-deoxy-octanoic acid) which is responsible for primary poisoning, therefore the effectiveness of cocoa parasitic leaf extract is more inhibited *Shigellady senteriae* compared to *Salmonella typhi*[12].

4. CONCLUSION AND SUGGESTIONS

Based on the results of the study about the effect of cacao leaf parasite extract (*Dendrophthoe pentandra* (L.) Miq) on the growth of *Shigella dysenteriae* and *Salmonella typhi* bacteria, the effective concentration in *Shigella dysenteriae* was shown at a dose of 12% and 16% in *Salmonella typhi.*

Based on the research, here are some suggestions from the researcher:

a. Further testing is needed on the effect of cocoa parasite leaf extract (*Dendrophthoe pentandra* (L.) Miq) on the growth of *Shigella dysenteriae* and *Salmonella typhi* bacteria using in vivo.
b. Further research needs to be done on the different types of solvents against the antibacterial properties of the cocoa leaf parasite extract (*Dendrophthoe pentandra* (L.) Miq).

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c. It is necessary to test the content of cacao leaf parasite extract compounds (*Dendrophthoe pentandra* (L.) Miq) to determine the percentage of compounds inside.

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