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COMPOSING THE MIXTURE OF *A.CORDIFOLIA* LEAF EXTRACT IN ELIMINATING *SALMONELLA TYPHI* AND *STAPHYLOCOCCUS AUREUS* BACTERIA

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Abstract

Background: *A.cordifolia* leaf is one of the traditional medicinal plants that have many benefits, including cough medicine, diabetes, shortness of breath, burns, post-operative wounds, dysentery, kidney inflammation, inflammation of the intestine, acne, bleeding gums, smoothing menstruation, increase appetite, and increase endurance. This research aims to find out the minimum concentration of *A.cordifolia* leaf extract that was able to kill bacteria *Salmonella typhi*

Methods: This research is an experimental laboratory study with a research design using a Completely Randomized Design (CRD) in which the treatment of test bacteria is carried out with variations in concentration and contact time of the *A.cordifolia* leaf extract and *A.cordifolia* leaf using alcohol extract. The controlled variable was the concentration of *A.cordifolia* leaf extract with contact time variation while the response variable was the result of bacterial colony growth. For negative control applied sterilized aqua (0%) and as a comparison chloramphenicol was used.

Result: The results showed that *A.cordifolia* which extracted by ethanol solvent killed bacteria *S. Typhi* at a concentration of 80% with a contact time of 10 minutes, and bacteria *S. aureus* at a level of 100% with a contact time of 10 minutes, whereas A.cordifolia water extract could kill bacteria *S.typhi* at a concentration 80% with a contact time of 20 minutes, and bacteria *S. aureus* at a concentration of 100% with a contact time of 20 minutes.

Conclusion: *A.cordifolia*, which extracted by ethanol solvent, has a stronger killing power than one extracted with water solvent.

Keyword: A.cordifolia leaves, Salmonella typhi, Staphylococcus aureus, extraction

Introduction:

In this modern era, people's lifestyles which pay less attention to environmental cleanliness have resulted in the emergence of infectious diseases. Nowadays, the motto of *back to nature is* often touted, ranging from living behavior, eating patterns, to the treatment of diseases. In curing various diseases, medicinal plants have become a demand that is much in market by the community, because besides being safe, the costs incurred are relatively inexpensive compared to drugs that are based on synthesis. The use of chemical medicines has side effects that can be either direct or indirect side effects or accumulate. This happens because chemicals are inorganic and pure while the body is organic and complex. Then chemicals are not materials that are genuinely suitable for the body. The use of chemicals in the body is considered as something that is unavoidable and is of limited use that can be accepted and tolerated by the body.

In various regions in Indonesia, many plants can be used for the treatment of multiple diseases, one of which is A.cordifolia (*Anredera cardifolia*). The content in the *A.cordifolia* leaves antioxidant activity, ascorbic acid, and high total phenols.[1] These substances can fight Gram-positive bacteria such as *Staphylococcus aureus*. And able to also fight six Gram-negative bacteria such as *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Enterobacter aerogenes.*[2]

A.cordifolia leaves contain a variety of active substances, namely alkaloids, saponins, flavonoids and polyphenols which have antibacterial power.[3] In Indonesia, bacterial infectious diseases are still a health problem, especially in areas with poor sanitation and individual hygiene. Then it is necessary to develop a natural material that can be used as an

alternative treatment, one of which is *A.cordifolia* leaves. The use of *A.cordifolia* leaves in the community for treatment has long been used, but scientific research needs to be done to show the efficacy and the right dose. An antibacterial activity test of ethanol extract and *A.cordifolia* leaf water extracts has been conducted to kill *Salmonella typhi* and *Staphylococcus aureus* by determining the minimum concentration with contact time variation using serial dilution.

The method In general, *A.cordifolia* leaves can accelerate health recovery after surgery, childbirth, circumcision, all internal injuries, intestinal inflammation, smooth and normalize circulation and blood pressure, prevent *strokes*, ulcers, gout, increase and restore vitality to the immune system, vizier (hemorrhoid), smooth urination, bowel movements, diabetes, typhoid fever, back pain, and others.[4]

Typhoid fever is a contagious disease in the community caused by infection with bacteria *Salmonella typhi*. Typhoid fever is found in many countries, or areas that do not maintain cleanliness both water and food. The highest typhoid fever infection is often found in children [5]. Infection can occur through the mouth, from eating and drinking contaminated with bacteria *Salmonella typhi*. Foods that are usually tainted include cakes containing milk sauce, minced meat, poultry sausages, and eggs. Although infectious and sick people can contaminate food and drink, the source of infection by bacteria *Salmonellosis* is low-level animals. Many species of bacteria *Salmonella* occur naturally in chickens, turkeys, ducks, rodents, cats, dogs, and many other animals. These individuals can extract millions of bacteria *Samonella* in their stools which are sources of food and drink pollutants either directly or through flies as vectors. Typhoid fever if not treated immediately, can cause death of 10-20%. Because there is an *ulcer* in the small intestine, where intestinal perforation, intestinal bleeding, toxemia, and other complications occur.

Based on the background above in previous studies, the minimum concentration of *A.cordifolia* leaf extract that was able to kill bacteria *Salmonella typhi* was at an extract concentration of 60% with a contact time of 60 minutes and in bacteria *S. aureus* was a concentration of 100% with a contact time of 90 minutes, while the minimum concentration of *A.cordifolia* leaf juice that can kill bacteria *Salmonella typhi* is at 80% concentration with a contact time of 180 minutes, and the bacteria *S. aureus* is 100% with a contact time of 60 minutes. Thus, further research was conducted to determine the lowest concentration of ethanol extract and *A.cordifolia* leaf water extract which can kill bacteria *Salmonella typhi* and *Staphylococcus aureus*.

METHODS

This study was an experimental laboratory study with a research design using a Completely Randomized Design (CRD) in which the treatment of test bacteria was carried out with variations in concentration and contact time of the *A.cordifolia* leaf Extract and Alcohol Extract. The controlled variable was the concentration of *A.cordifolia* leaf extract with contact time variation while the response variable was the result of bacterial colony growth. For negative control using sterile aquades (0%) and as a comparison used chloramphenicol.

RESULTS

The results of the ethanol extract of *A.cordifolia* leaves against bacteria *S.typhi*, and compared with controls showed that at 80% concentration with a contact time of 10 minutes, ethanol extract was still able to kill *S.typhi*. Whereas at a concentration of 60% with all variations of contact time, there was no killing power against *S.typhi,* which was marked by the growth of bacterial colonies on SS agar media (Table 1).

Table 1: Testing of Ethanol Extract of A.cordifolia leaves against *S.typhi* with contact time variations

Concentration of *A.cordifolia* Extract (%)	*CONTACT TIME (MIN)*								CONTROL POSITIVE	CONTROL NEGATIVE
	10		20		30		40			
	1	2	1	2	1	2	1	2		
	-	-	-		-		-		+	-
60	-	-	-		-		-			
80		+	+		+		+			
100		+	+		+		+			

For the test results of *A.cordifolia* leaf water extract against bacteria *S.typhi*, and compared with controls it was seen that water extract at a concentration of 80% with a contact time of 10 minutes, could not kill *S.typhi*. Their killing power is only seen at 80% concentration with a contact time of 20 minutes. While at a concentration of 60% with all variations of contact time, there is no ability to kill against *S.typhi,* which is characterized by the growth of bacterial colonies on SS agar media. (Table 2)

Table 2: Testing the extract of water leaves against *A.cordifolia S.typhi* with contact time variations

Concentration (%)	*CONTACT TIME (MINUTE)*								POSITIVE	CONTROLS NEGATIVE CONTROLS
	10		20		30		40			
	1	2	1	2	1	2	1	2		
40	-	-	-	-	-	-	--	--	+	-
60	-	-	-	-	-	-	-	-		
80	-	-	+	+	+	+	+	+		
100	+	+	+	+	+	+	+	+		

The results of the ethanol extract of *A.cordifolia* leaves against the bacteria *S.aureus* using Mannitol Salt Agar germination media and compared with controls showed that at a concentration of ≤80% with all contact time, ethanol extract could not kill *S.aureus*. The killing power of *S. aureus was* only seen at a concentration of 100% with a contact time of 10 minutes, which was characterized by the absence of growth of bacterial colonies on MSA media (Table 3).

Table 3: Testing of ethanol extract of A.cordifolia leaves against *S. aureus* with the variation of contact time

Concentration (%)	*CONTACT TIME (MINUTE)*								POSITIVE	CONTROL NEGATIVE CONTROL
	10		20		30		40			
	1	2	1	2	1	2	1	2		
40	-	-	-	-	-	--	--	--	+	-
60	- -	-	-	-	-	-	-	-		
80	-	-	-	-	-	-	-	-		
100	+	+	+	+	+	+	+	+ +		

The test results of *A.cordifoli* leaf water extracted with a variation of contact time against the bacteria *S.aureus* and compared with control showed that at a concentration of ≤80% with all contact time, and a concentration of 100% with a contact time of 10 minutes the water extract could not kill *S.aureus*. The killing power of *S. aureus is* only seen at a concentration of 100% with a contact time of 20 minutes, which is characterized by the absence of bacterial colony growth on MSA media (Table 4).

Table 4: Testing the extract of A.cordifolia leaf water against *S.aureus* with variations in contact time

Concentration (%)	*CONTACT TIME (MINUTE)*								POSITIVE	CONTROL NEGATIVE CONTROL
	10		20		30		40			
	1	2	1	2	1	2	1	2		
40	-	-	-	-	-	-	--	--	+	-
60	- -	-	-	-	-	-	-	-		
80	-	-	-	-	-	-	-	-		
100	-	-	+	+	+	+	+	+ +		

DISCUSSION

Biological activity of water-soluble flavonoid compounds against bacteria by destroying the cytoplasmic membrane of bacteria consisting of amino acids and lipids and reacting them with alcohol groups found in flavonoid compounds.[6] From this process will cause cell walls damaged and compounds into the bacterial cell nucleus. Furthermore, this compound will contact with DNA in the nucleus of bacterial cells and through differences in polarity between the constituent DNA lipids and alcohol groups in flavonoid compounds will occur reactions that damage the lipid structure of bacterial DNA so that it will undergo lysis and die.[7]

The damaged cell wall condition will immediately decompose, followed by penetration of phenol into

the bacterial cell and cause protein coagulation.[8] Besides, bioactive phenol compounds can also cause cell lysis and cause protein denaturation. Some saponins have a nature like soap which is a compound of"*Surfactant agent*" is durable and has a structure that can bind to hydrophilic molecules and organic molecules non-polar (lipophilic) to damage the cytoplasmic membrane and kill the bacteria.[9]

Additionally, obtained experimental results show that the antibacterial power of ethanol extract is higher than the anti-bacterial power of water extract. This is because the content of active compounds in ethanol extracts and water extracts is different, namely in ethanol extracts more energetic compounds are dissolved/extracted in ethanol solvents compared to water extracts, such as flavonoid compounds, polyphenols are more easily extracted in ethanol solvents, so the power the antibacterial properties of ethanol extract are more significant than the anti-bacterial power of water extracts.[10]

The results of previous study showed that *A.cordifolia* leaf extract had a minimum kill concentration of 50% against *S.aureus.* The previous study concluded that *A.cordifolia* (Anredera cordifolia) leaf extracts with concentrations of 3%, 5%, 7%, 9%, 11%, and 13% were only bacteriostatic, which inhibited the growth of bacteria *Vibrio harveyi* with the best concentration obtained, which was 13 %.[2] Furthermore, the results previous research showed that the extract of A.cordifolia leaves at 75% concentration was the most effective concentration as an antibacterial against *E. coli*, followed by a concentration of 50% and a concentration of 25%.[11]

A.cordifolia leaf extract has the ability to inhibit the growth of bacteria *Propionibacterium acnes* seen by the inhibition zone formed. The most effective concentration to inhibit the growth of Propionibacterium acnes is at a concentration of 100% at 9.00 mm at 24 hours and 11.20 mm at 48 hours.[12] An extract of *A.cordifolia* leaves at a concentration of 60% with a contact time of 60 minutes can kill *S.typhi*, and at 100% with a contact time of 90 minutes. Can kill bacteria *S. aureus* while *A.cordifolia* leaf juice at 80% concentration with 180 minutes contact time can kill *S.typhi*, and at 100% concentration with 60 minutes contact time can kill the bacteria *S.aureus*

CONCLUSION

A.cordifolia leaf extract (Ethanol and Water) has antibacterial activity and can kill the bacteria *Salmonella typhi* and *Staphylococcus aureus.* The minimum concentration of Ethanol Extract of *A.cordifolia* leaves that can kill the bacteria *S. Typhi* is 80% with a contact time of 20 minutes and the bacteria *S. aureus* is 100% with a contact time of 10 minutes The minimum concentration of water extract of *A.cordifolia* leaves that is capable of killing the bacteria *S. Typhi* is on 80% concentration with contact time of 10 minutes, and the bacteria *S.aureus* are 100% with contact time of 20 minutes. *A.cordifolia* leaf extract with ethanol solvent has stronger antibacterial activity than *A.cordifolia* extract with water solvent.

DECLARATION

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