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The feasibility of tannin containing in *Averrhoa Bilimbi* leaves as alternatives for antiseptics

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Abstract
Postpartum infection in perineal wounds is a problem during childbirth. The infection supports maternal mortality and morbidity in Indonesia 38%. The use of antiseptics to inhibit bacterial growth and prevent infection, namely 10% povidone-iodine has several side effects, one of which is allergic, thus encouraging research to find safer and more effective herbal ingredients. The research to prove the tannins on the leaves of star fruit (*Averrhoa bilimbi leaf*) as the inhibitor or the growth of bacteria *S. haemolyticus α, β, S. aureus*, and *E. Coli*. Subjects of the study were bacteria *S. haemolyticus α, β, S. aureus,* and *E.coli*. The design of the study was a completely randomized design, inhibitory test using disc diffusion method *Kirby-Bauer* with *One Way ANOVA* and *Post Hoc Tukey HSD.* The inhibitory power of bacterial growth *S. haemolyticus, S. aureus* and *E. coli* in tannins 1% and 10% are no better than iodine control 10%, in tannin 20% the growth inhibitory ability of bacteria were the *S. haemolyticus α* and *S. aureus* same as iodine control 10%, while *S. haemolyticus β* and *E. coli* were better than 10% iodine, namely *S. haemolyticus α* tannin 20% = 16.83 mm and control = 15.67 mm with p = 0.087 (> 0.05) not significantly different. *S. haemolyticus* β tannin 20% = 17 mm and control = 15.67 mm with value p = 0.035 (<0.05) was significantly different, *S. aureus* tannin 20% = 17 mm and control = 16 mm with p = 0.131 (> 0.05) was not significantly different and *E. coli* tannin 20% = 16.83 mm and control = 15 mm with a value of p = 0.002 (<0.05) was significantly different. The 20% tannin concentration is a significant concentration in inhibiting the growth of bacteria *S. haemolyticus α, β, S. aureus,* and *E.coli* so that it can be used as an antiseptic alternative to perineal wounds.

Keywords: postpartum infection, antiseptic, alternative, tannin, *Averrhoa bilimbi* leaf, bacterial inhibitory power

1. Introduction
The postpartum period is the time after the baby is born followed by the birth of the placenta and fetal membranes until the return of the female reproductive tract in nonpregnant conditions [1]. Problems that arise during childbirth are infections *postpartum* in perineal wounds such as vulvitis, vaginitis, cervix, and endometritis. The quality of the perineal injury is said to be good if there are no signs of infection, namely, red, swollen, hot, painful and functional [2]. *Postpartum* infection still plays a role as a cause of maternal death, especially in developing countries such as Indonesia [3].

About 80% of maternal deaths are due to increased complications during pregnancy, childbirth and after childbirth, one of which is the infection of *postpartum* [4]. According to the Indonesian Ministry of Health in 2008, the most significant cause of maternal deaths that occurred in Indonesia during the period was *postpartum* bleeding 28%, eclampsia 24%, infection 11% and others by 11%. Infection of *Postpartum* supports high maternal mortality and morbidity in Indonesia, which is about 38% of the total *postpartum* mothers. The incidence of disease of *postpartum* in Indonesia contributes 10% to direct obstetric causes and 8% of all maternal deaths [5].

Based on the results of Nanda Juita's research (2011) the factors that influence the occurrence of *postpartum* infection or the most significant perineum wound healing are hygiene [6]. Through wound healing management, sanitation is done to reduce the amount of bacterial proliferation, kill bacteria, prevent infection, and overcome diseases. *Postpartum* infection is mainly caused by *Streptococcus haemolyticus*, *Staphylococcus aureus*, *Escherichia coli* and *Clostridium welchii* (rarely found) [7]. Whereas according to Ahmed *et al*. (2015) bacteria present in puerperal infecies are *Escherichia coli, Streptococcus haemolyticus, Proteus mirabilis, Staphylococcus aureus*, and *Klebsiella pneumonia* [8].

According to Rifzul's research (2017), an infection can be prevented by *vulva hygiene* (cleansing the vulva area). *Vulva hygiene* is an action aimed at reducing discomfort, cleanliness, preventing disease and accelerating wound healing. With *vulva hygiene* can also pay attention to the condition of the mother's perineum, if abnormalities arise, treatment can be done immediately so that rapid healing occurs [9]. In addition to *vulva hygiene*, wounds are also treated by giving conservative antiseptic fluid to infected contamination wounds and infected wounds to inhibit bacterial growth. Among types of sterile liquids namely liquid iodine, wound care in general usually use 10% povidone-iodine, but iodine use has some adverse side effects, such as skin allergies and too much iodine can slow the wound granulation process, iodine is also irritating and more toxic when it enters the blood vessels, causing systemic effects due to tissue anoxic shock [10].

The ideal antiseptic characteristic is to kill microorganisms in a wide range, remain effective against various kinds of dilution, non-toxic to the human body, not easily cause sensitivity reactions, both local and systemic, react quickly, work efficiently even though it consists of organic and durable

ingredients [11]. One of the natural ingredients that can be used as an antiseptic alternative is starfruit leaves (*Averrhoa bilimbi leaf*). Another advantage of herbal ingredients is its wide availability, such as star fruit leaves which are easy to find in Indonesia [12].

Starfruit leaves (*Averrhoa bilimbi leaf*) is one of the plants used as natural medicine; starfruit leaves contain active compounds which function to inhibit bacterial growth [13]. Starfruit leaves are used as traditional medicine because there are active substances which can inhibit the growth of bacteria called antiseptic substances [14].

This research was carried out using laboratory tests because taking only tannin compounds on starfruit leaves as an antiseptic alternative, is an innovation that must be tested first with in vitro (enzymes, cells or microorganisms in glassware) according to computational rules for drug manufacturing [15]. This study aims to explore information about the difference in inhibitory concentration on the content of tannin starfruit leaves compounds on the growth of bacteria that cause *postpartum i*nfection.

2. Materials and methods

This study is *a posttest only control group design* using Completely Randomized Design (CRD) with 4 treatments and 6 repetitions. The treatment is with the concentration of tannin 1%, 10%, 20% and the control is povidone iodine 10%. The number of replications / repetitions in this experiment is calculated by Federer's formula as follows:

$$(n - 1)(t - 1) \geq 15$$

Description:
n = number of replications
t = number of treatments
So,

$$(n-1)(4-1) = 15 >> (n-1)(3) = 15 >> n - 1 = 5 >> n = 5 + 1 = 6$$

So the number of replications done in this study was 6 times. Replication was carried out on each test bacteria, namely *S. haemolyticus, S. aureus* and *E. coli*. The research design is as follows:

Treatment	Replication					
	A	B	C	D	E	F
P1	P1A	P1B	P1C	P1D	P1E	P1F
P2	P2A	P2B	P2C	P2D	P2E	P2F
P3	P3A	P3B	P3C	P3D	P3E	P3F
P4	P4A	P4B	P4C	P4D	P5D	P4F

Table 1: Research Design Description

P1: Tannin Concentration 1%	A : Replication 1
P2: Tannin concentration 10%	B: Replication 2
P3: Tannin concentration 20%	C: Replication 3
P4: Povidone Iodine 10%	D: Replication 4
	E: Replication 5
	F: Replication 6

The process of data analysis used is the normality test using *Saphiro Wilk* to see whether the data obtained is typically distributed with a value of $p > 0.05$.

Then if the data is not normally distributed and then the *Kruskal Wallis test is* then performed *Mann Whitney U Test* to determine whether or not there is a significant difference between treatment groups [16-18].

3. Results & Discussion

After researching tannin potential in starfruit leaves as an alternative antiseptic, inhibitory tests on *S. haemolyticus, S. aureus* and *E. coli bacteria* obtained the following results:

Table 2: Diameter of Inhibitory Zone (bacteria *S. Haemolyticusα*)

Treatment Group	Repetition / Replication(mm)						Mean
	1	2	3	4	5	6	
P_1	5	4	3	3	3	4	3.67
P_2	10	11	10	9	9	9	9, 67
P_3	16	18	17	16	17	17	16.83
P_4	15	16	15	17	15	16	15.67

Table 2 shows the zone diameter of growth inhibition of bacteria *S. haemolyticus α* at 1% tannin concentration was 3.67 mm with the lowest inhibition zone 3 mm and zone highest inhibition of 5 mm. At 10% tannin concentration the average inhibition zone was 9.67 mm with the lowest inhibition zone 9 mm and the most upper inhibitory region 11 mm, whereas in the tannin concentration of 20% the average inhibition zone is 16.83 mm with the lowest inhibition zone 16 mm and the highest inhibitory zone of 18 mm.

Table 3: The diameter of Inhibitory Zone (bacteria *S. haemolyticus β*)

Treatment Group	Repetition / Replication(mm)						Mean
	1	2	3	4	5	6	
P_1	3	5	4	5	4	4	4,.7
P_2	10	9	9	10	11	11	10
P_3	18	17	16	17	17	17	17
P_4	15	15	15	16	16	17	15.67

The table showed that there were differences in mean diameter of bacterial inhibition zone *S. haemolyticus β* with 1% tannin concentration was 4.17 mm with the lowest inhibitory zone of 3 mm and the highest inhibitory zone of 5 mm. At 10% tannin concentration, the average inhibition zone was 10 mm with the lowest inhibition zone 9 mm and the most top inhibitory area 11 mm. Whereas in the 20% tannin concentration the average inhibitory zone was 17mm with the lowest inhibition zone 16 mm and the highest inhibition zone of 18 mm.

Table 4: The diameter of Inhibitory Zones (Bacteria *S. aureus*)

Treatment Groups	Repetition / Replication(mm)						Mean
	1	2	3	4	5	6	
P_1	4	6	4	5	5	6	5
P_2	12	10	10	10	11	11	10, 67
P_3	17	17	17	16	17	18	17
P_4	16	16	17	16	16	15	16

Table 4 shows that there is a difference in the mean (mean) Diameer of the inhibitory zone of bacteria *S. aureus* with 1% tannin concentration is 5 mm with the lowest inhibition zone 4

mm and zone highest inhibition of 6 mm. At 10% tannin concentration, the average inhibition zone was 10 mm with the lowest inhibition zone 9 mm and the most top inhibitory area 11 mm. Whereas in the 20% tannin concentration the average inhibitory area was 17mm with the lowest inhibition zone 16 mm and the highest inhibition zone of 18 mm.

Table 5: The diameter of Inhibitory Zone (Bacteria E. coli)

Treatment Group	Repetition / Replication(mm)						Mean
	1	2	3	4	5	6	
P_1	4	4	3	3	5	4	3.83
P_2	11	12	11	10	10	10	10, 67
P_3	17	17	16	16	17	18	16.83
P_4	15	14	15	15	15	16	15

Table 5 showed that there was a difference in the mean diameter of the inhibition zone *E. coli* with 1% tannin concentration was 3.83 mm with the lowest inhibition zone 3 mm and the highest inhibitory zone of 5 mm. At 10% tannin concentration, the average inhibitory region was 10.67 mm with the most depressed inhibitory region of 10 mm and the highest inhibitory area of 12 mm. Whereas in the tannin concentration of 20% the average inhibition zone is 16.83 mm with the lowest inhibition zone 16 mm and the highest inhibitory zone of 18 mm.

The results of the research data are in the form of inhibition zone diameter of bacterial growth analyzed using the parametric test *One Way A*NOVA continued with the tests *Post Hoc* and *Tukey HSD*. Parametric test requirements *One Way ANOVA* are normal data distribution. For this reason, the data normality test and homogeneity test are carried out first. For normality *Shapiro Wilk* in all bacterial groups, the value $p > 0.05$ showed that the growth inhibition zone data on all bacteria and in all treatment groups were normally distributed.

Homogeneity of variance between groups tests obtained by value $p > 0.05$, which indicates that the data variance between groups on the inhibition of bacteria is homogeneous.

The analysis of the test *One Way A*NOVA obtained $p = 0{,}000$ (<0, 05) which showed differences between treatment groups or differences in size of inhibitory zones in each group. For this reason, the test is required *Post Hoc Test* and *Tukey HSD.*

Table 6: Results of Test of *Post Hoc* Bacterial Growth *S. haemolyticus αInhibition*

Group	Group	Sig.	Group	Group	Sig.
Tannins 1%	Tannin 10%	.000	Tannins 20%	Tannins 1%	.000
	Tannins 20%	.000		Tannins 10%	.000
	Iodine 10%	.000		Iodine 10%	.087
Tannins 10%	Tannins 1%	.000	Iodine 10%	Tannins 1%	.000
	Tannin 20%	.000		Tannins 10%	.000
	Iodine 10%	.000		Tannins 20%	.087

Based on table 6. The results of the analysis of the test *Post Hoc* showed that there was a significant difference in the 1% tannin group and 10% tannin compared to 10% iodine with $p = 0.000$ (<0.05). Whereas in the 20% tannin group and 10% iodine the value of $p = 0.087$ (> 0.05) which means that there are no significant differences between the two groups. Then to see the mean or average for each group, the test was conducted *Tukey HSD*.

Table 7: Test Result of *Tukey HSD* Bacterial Inhibition Zone *S. haemolyticus α*

Group	Subset for alpha = 0.05		
	1	2	
Tannin 1%	3.6667		
Tannin 10%		9.6667	
Iodine 10%			15.6667
Tannin 20%			16.8333
Sig.	1.000	1.000	.087

The results of the test analysis *Tukey HSD* show that the mean value in the subset column has different values for each group. But in the 20% tannin group and 10% iodine the mean value is in one column of the same subset with a value that is not much different. This mean value is not much different which causes the 20% tannin group, and 10% iodine is not significantly different.

Analysis of *Post Hoc* showed no significant difference in all groups tannins 1%, 10% and 20% compared with 10% iodine with $p = 0.000$ and 0.035 (<0.05). The analysis of the *Tukey HSD test* shows that the mean values in the subset column have different values and in separate columns for each group. This indicates that all groups differ significantly.

For bacteria *S. aureus* The results of the analysis of the test *Post Hoc* showed that there was a significant difference in the 1% tannin group and 10% tannin compared to 10% iodine with $p = 0.000$ (<0.05). Whereas in the 20% tannin group and 10% iodine value $p = 0.131$ (> 0.05), which means that there are no significant differences between the two groups. Then the results of the analysis of the *Tukey HSD test* showed the mean value in the subset column showed different values for each group. However, in the 20% tannin group and iodine 10%, the mean value was in one column of the same subset with values not much different. This mean value is not much different which causes the 20% tannin group, and 10% iodine is not significantly different.

For the body of *E. coli***,** the results of the analysis of the *Post Hoc Test* showed that there were significant differences in all groups with $p = 0.000$ and 0.002 (<0.05). Then the results of the analysis of the *Tukey HSD test* showed that the mean value in the subset column had different values and in the various columns of each group. This indicates that all groups differ significantly.

Tannin extract from starfruit leaves was proven to inhibit the growth of *S. haemolyticus*, *S. aureus*, and *E. coli bacteria.* The higher the tannin concentration, the higher the inhibitory effect on the bacteria. The potential of tannins to inhibit the growth of bacteria *S. haemolyticus α, β, S. aureus*, and *E. coli* showed that at 20% tannin concentration the highest inhibitory power was 16-20 mm and was effective in inhibiting growth. Bacteria *S. haemolyticus α, β, S. aureus* and *E. coli* [19].

The inhibitory power of bacteria on tannin concentration is 20% better than the control of 10% povidone-iodine when viewed from the mean value or average inhibitory power which shows moderate inhibitory power, this is influenced by the high tannin concentration which is consistent with the results of this study, namely the higher tannin concentration, the higher the inhibitory power of bacteria. This is in accordance with Affandi's (2012) study which showed that the higher the level of povidone-iodine, the higher the inhibitory

power and killing power of MRSA and MSSA bacteria [20], as well as the research of Sulistiyaningsih (2010) which showed that at minimum povidone inhibitory concentration iodine which starts from 0.1% and 0.2% earned bacterial growth *P. aeruginosa* and *multiresistant P.*aeruginosa, whereas at concentrations of 0.3% - 0.8% is not found bacterial growth [21].

The results of this study are the tannin potential of starfruit leaves on the inhibitory power of *S. haemolyticus, S. aureus* and *E. coli bacteria in* accordance with previous studies, namely in the study of Sonia Saini (2016) chloroform extract of star fruit leaves showed activity antibacterial against gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermis*. Starfruit leaf extract can also inhibit the growth of fungi such as *Candida albicans* with a minimum concentration value of 15.65-62.50 µg / ml [22-23]. In the study, K. Ashok Kumar *et al*. (2013) chloroform extract of 100 µg / mL starfruit leaves showed activity antibacterial against gram-positive bacteria such as *B. cereus* and gram-negative bacteria such as *S. Typhi* [24].

In this study, it was proved that the tannin compounds of starfruit leaves were active as antibacterial/antiseptic, so tannin has the potential as a sterile alternative for the prevention of infection in maternal perineal wounds *postpartum*.

This is consistent with previous tannin studies using different plants, namely in the study A. Doos *et al*. (2009) showed that tannin extract from leunca leaves (*Solanum trilobatum Linn*) could fight microorganisms such as *S. aureus, S. pyrogens, S. Typhi, E. coli, Proteus vulgari* and *Pseudomonas aeruginosa* with a minimum inhibitory concentration of 2.0 mg/ml and a minimum suicide concentration between 1.5 and 2.0 mg / ml [25]. In the study of Rodrigues *et al*. (2014) showed that tannins from Brazil guava leaves (*Psidium guineense*) could inhibit the growth of bacteria *S. aureus* with a minimum inhibitory concentration of 256 µg / ml and bacteria *Pseudomonas aeruginosa* with a minimum inhibitory concentration of 512 µg / ml [26].

Stannin compounds can form complexes with proteins through hydrophobic interactions so that in the presence of hydrophobic bonds there will be denaturation and ultimately disrupted bacterial cell metabolism 18. Increased permeability of bacterial walls is followed by intracellular leakage which causes the cell component to exit so that the activity physiological of bacterial cells decreases and causes inhibition of bacterial growth [27].

4. Conclusions

Effective concentration on tannins which can inhibit the growth of *S. haemolyticus, S. aureus* and *E. coli bacteria* is 20% tannins. It was proved by the mean results in the *Tukey HSD test* that the bacteria *S. haemolyticus* α = 16, 8333, *S. haemolyticus* β = 17.0000, *S. aureus* = 17.0000 and *E. coli* = 16.8333 which showed the strength of the medium inhibitory power.

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