The Inhibition Test of Red Betel Leaves (*Piper crocatum* Ruiz & Pav) Extract Against the Growth of *Streptococcus pneumoniae* and *Salmonella typhi*

Evi Kurniawaty[1,*], Herina Azzahra[1], Intanri Kurniati[1], Waluyo Rudiyanto[1], Aristianto Husin[1] and Silvia Andriani[2]

[1]*Faculty of Medicine, University of Lampung, Indonesia*

[2]*Faculty of Health, University of Muhammadiyah Pringsewu Lampung, Indonesia*

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Abstract: Infectious diseases are still a health problem in the world, both in developed and developing countries. Infectious disease is a disease caused by pathogenic microbes (viruses, bacteria, fungi) and is the main cause of high morbidity and mortality rates. Respiratory tract infections and typhoid fever caused by bacteria are still the most common health problems. Pneumonia is an infection or acute inflammation of the lung tissue caused by various *Streptococcus pneumoniae* bacterial microorganisms, exposure to chemicals or physical damage to the lungs. Meanwhile, typhoid fever is a type of infection caused by the bacterium *Salmonella Enterica*, especially the *Salmonella Typhi* derivative. The high level of resistance to antimicrobials, which currently still causes chronic infections in humans, has led to the idea that medical personnel must be more careful in using and finding the right antibiotics. One of the potential medicinal plants that is known empirically to have properties for curing various diseases is red betel leaf (*Piper crocatum ruiz* and Pav.). This study aims to prove that red betel leaves has an inhibitory effect against *Streptococcus pneumoniae* and *Salmonella typhi*. This study used an experimental method. The technique used to measure antibiotic activity is the method of well diffusion and continued by Kruskal-Wallis test and Mann-Whitney test. The results showed that extract of red betel leaf can inhibit the growth of *Salmonella typhi* at concentrations 20%, 30%, 40%, and 50% and the most effective is 50% concentration. There is no result on *Streptococcus pneumonia.* Red betel leaves (*Piper crocatum Ruiz & Pav.)* have an inhibitory power against *Salmonella typhi* but does not have an inhibitory effect on *Streptococcus pneumoniae* bacteria.

1 INTRODUCTION

Infectious diseases are diseases caused by pathogenic microbes (viruses, bacteria, fungi) and are the main cause of high morbidity and mortality rates. Respiratory tract infections and typhoid fever caused by bacteria are still the most common health problems (Syahidi HM, 2013).

Pneumonia is known to attack around 450 million people every year. In 2018, the prevalence of pneumonia based on health worker diagnosis was around 2%, whereas in 2013 it was 1.8%, which shows an increase (Riskesdas, 2018). The number of pneumonia sufferers in Indonesia in 2013 ranged from 23% to 27% and deaths due to pneumonia were 1.19%. In 2010 in Indonesia, pneumonia was included in the top 10 inpatient diseases in hospitals with a crude mortality rate (CFR) or the death rate for a particular disease in a certain period of time divided by the number of cases, namely 7.6% (Ministry of Health, 2019).

Meanwhile, typhoid fever still has the highest incidence of transmission in children, especially in endemic countries. If you look at Indonesia's health profile data for 2011 and 2012, typhoid fever is included in the top 10 diseases with the most hospitalized patients, namely in 3rd position. In 2011 there were 80,850 cases with 1,747 deaths. In 2012 the number of cases decreased to 41,081 cases with 274 deaths (Ministry of Health of the Republic of Indonesia, 2013).

The high level of resistance to antimicrobials, which currently still causes chronic infections in

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humans, has given rise to the opinion that medical personnel must be more careful in using and finding the right antibiotics. This also encourages people, both medical and non-medical personnel, to use medicinal plants as an alternative healing method. One of the potential medicinal plants which is known empirically to have properties for curing various diseases is Red Betel Leaf (*Piper crocatum* Ruiz and Pav.). In society, red betel is famous for its properties in treating diseases such as diabetes mellitus, hepatitis, kidney stones, lowering cholesterol, preventing stroke, gout, hypertension, coronary heart disease, uterine cancer, breast cancer, hemorrhoids, tuberculosis, medicine. toothache, canker sores, body odor, venereal disease, liver inflammation, prostate inflammation, eye inflammation, vaginal discharge, ulcers, fatigue, joint pain, softening of the skin, ear inflammation, cough medicine, pneumonia, sore throat, gingivitis, inflammation breasts, nose bleeds, and coughing up blood. Apart from that, there has been a lot of research on antimicrobial plants that can fight infection, one of which is red betel leaf (*Piper crocatum* Ruiz and Pav.) and green betel leaf (*Piper betle* Linn) (Julistiara, 2017).

2 METHOD

This research is an experimental study using a Post-test Only Control Group design. In this study, the experimental group was given treatment for a certain period of time, then the dependent variable was measured in the two groups, and the results were compared for differences. The test microbes used were gram-positive bacteria (+) *Streptococcus pneumoniae* and gram-negative bacteria (-) Salmonella typhi obtained from the Bandar Lampung Regional Health Laboratory (LABKESDA).

The research test material used was red betel leaves (*Piper crocatum* Ruiz & Pav.) which were washed using clean water, then dried using an oven at a temperature of 105°C. Extraction was carried out by the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences (FMIPA), University of Lampung using the soxhletation method with 96% ethanol solvent at a temperature of 70°C. Then the red betel leaf extract is diluted using saline to obtain varying concentrations.

The culture medium used in this study was blood agar plates in 10 cm petri dishes. This medium is used to culture *Streptococcus pneumoniae* and *Salmonella typhi*. After culturing, Mueller-Hinton Agar (MHA) media was used as a medium for testing the diameter of the inhibition zone for *Streptococcus pneumoniae* and *Salmonella typhi* bacteria.

The population in this study, namely pure culture *Streptococcus pneumonia* and *Salmonella typhi* bacteria, was divided into seven research groups. In this research, various levels of red betel leaf extract will be tested, namely at a level of 10%; 20%; 30%; 40%; and 50%, as well as with Erithromycin as a positive control for *Streptococcus pneumoniae* bacteria, Ciprofloxacin as a positive control for *Salmonella typhi* bacteria, and distilled water as a negative control. The research results were then tested for statistical analysis using a statistical program.

3 RESULTS

3.1 Phytochemical Test Results of Red Betel Leaves (*Piper crocatum* Ruiz and Pav.)

Based on the phytochemical test, the results showed that red betel leaves were identified as containing saponin, terpenoid, tannin and flavonoid compounds.

3.2 *Streptococcus pneumoniae* Bacterial Inhibition Zone Test

The results of measuring the inhibition zone of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract against *Streptococcus pneumonia* bacteria with 4 repetitions are as shown in the table 1.

Based on the data obtained, it is known that there is no inhibition zone formed from five concentrations of red betel leaf extract against *Streptococcus pneumoniae* in 4 repetitions. Meanwhile, in the K (+) group, the average inhibition zone was 19.625 mm and the K (-) group it was 0 mm.

Table 1: Results of measuring the diameter of the inhibitory zone of red betel leaf (*Piper crocatum* ruiz and Pav.) extract against *Streptococcus pneumoniae.*

Treament	Inhibition Zone Measurement *Streptococcus pneumoniae* (mm)				Inhibition Zone average (mm)
	I	II	III	IV	
K (+)	10,6	22,74	22,58	22,58	19,625
K (-)	0	0	0	0	0
P1	0	0	0	0	0
P2	0	0	0	0	0
P3	0	0	0	0	0
P4	0	0	0	0	0
P5	0	0	0	0	0

3.3 *Salmonella typhi* Bacterial Inhibition Zone Test

The results of measuring the inhibition zone of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract against *Salmonella typhi* bacteria with 4 repetitions, the measurement results obtained are presented in the table 2.

Table 2: Results of measuring the diameter of the inhibitory zone of red betel leaf extract (*Piper crocatum* Ruiz & Pav.) against *Salmonella typhi* bacteria.

Treatment	Inhibition Zone Measurement *Salmonella typhi* (mm)				Inhibition Zone average (mm)
	I	II	III	IV	
K (+)	37,68	38,7	42,42	42,92	40,43
K (-)	0	0	0	0	0
P1	0	0	0	0	0
P2	5,4	8,72	11,8	6,7	8,155
P3	8,50	8,42	8,42	9,38	8,68
P4	12,72	13,42	11,46	11,58	12,295
P5	15,98	14,92	13,98	13,04	14,48

Based on the table 2, it shows that there is an inhibitory power of red betel leaf extract against Salmonella typhi at concentrations of 20%, 30%. 40%, 50%. Based on the research results, the group that produced the largest inhibitory zone was the group with a concentration of 50%. This shows that the greater the concentration of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract, the greater the inhibition zone produced.

Next, the Shapiro-Wilk normality test was carried out to determine whether the data was normally distributed or not. The Shapiro-Wilk test was chosen because the data was less than 50. The results of the normality test are presented in the table 3.

Table 3: Normality test results of the inhibition zone of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract against *Salmonella typhi.*

Treatment	Sig.
K (+)	0,259*
K (-)	-
P1	-
P2	0,789*
P3	0,009*
P4	0,381*
P5	0,977*

Based on the table 3, in the normality test the significance results obtained for the K (+) group were 0.259, for the P2 group 0.789, for the P3 group 0.009, for the P4 group 0.381, and for the P5 group 0.977. Meanwhile, P1 and K (-) have no data. In the normality test, $p > 0.05$ was obtained, which means that all data was normally distributed.

Table 4. Results of the Levene homogeneity test of the inhibition zone of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract against *Salmonella typhi.*

Levene's homogeneity test	Sig.
Inhibitory zone of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract against *Salmonella typhi*	0,006

Based on the table 4, the homogeneity test obtained 0.006 ($p < 0.05$), which means the data is not homogeneous. Due to the non-homogeneity of the data, the requirements for carrying out the One- Way ANOVA Test were not met.

Table 5: Results of the Kruskal-Wallis test for the inhibition zone of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract against *Salmonella typhi*

Kruskal-Wallis test	**Sig.**
Inhibitory zone of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract against *Salmonella typhi*	0,0001*

Based on the results of the analysis, p value <0.05 was obtained which indicates that each treatment given with various concentrations of 10%, 20%, 30%, 40%, 50% and the positive control with erythromycin and the negative control using distilled water showed a significant difference in the zone. barriers are formed. Next, further analysis was carried out using the Mann-Whitney Post hoc Test to find out which groups were significantly different.

The Mann Whitney post hoc multicomparison test aims to determine whether there is a difference in the mean results of the inhibition zones formed in the 7 types of treatments. The difference in inhibition zone values is considered significant if $p < 0.05$. Based on the table above, it shows that there are significant differences in the inhibition zone at each concentration with the antibiotic Ciprofloxacin 500 mg. This shows that the inhibitory power of each concentration is not greater than that of the antibiotic used.

Table 6: Post hoc Mann-Whitney test of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract against *Salmonella typhi.*

Treatment	K (+)	K (-)	P1	P2	P3	P4	P5
K (+)	-	0,014*	0,014*	0,021*	0,020*	0,021*	0,021*
K (-)	0,014	-	1,000	0,014*	0,013*	0,014*	0,014*
P1	0,014*	1,000	-	0,014*	0,013*	0,014*	0,014*
P2	0,021*	0,014*	0,014*	-	0,772	0,083	0,021*
P3	0,020*	0,013*	0,013*	0,772	-	0,020*	0,020*
P4	0,021*	0,014*	0,014*	0,083	0,020*	-	0,043*
P5	0,021*	0,014*	0,014*	0,021*	0,020*	0,043*	-

4 DISCUSSIONS

Red betel leaf extract has an inhibitory response against Salmonella typhi bacteria but does not have an inhibitory effect against *Streptococcus pneumoniae* bacteria. This is caused by differences in the cell wall structure of the two bacteria. *Streptococcus pneumoniae* bacteria are gram-positive bacteria whose cell walls are composed of PG (peptidoglycan) so that the cell walls formed are quite thick and stiff. Meanwhile, Salmonella typhi bacteria are gram-negative bacteria whose cell walls contain much smaller amounts of PG (Peptidoglycan) than *Streptococcus pneumoniae*, but on the outside there is an outer membrane which is composed of lipoproteins and phospholipids and contains lipopilysaccharides. These bacteria have a thick cell wall in the form of peptidoglycan, which is located between the inner membrane and the outer membrane. This difference makes *Salmonella typhi* bacteria more susceptible to being destroyed by antibacterial agents than Streptococcus pneumoniae, so that in this study the inhibition zone for Salmonella typhi was larger than for *Streptococcus pneumoniae* (Pratiwi I, 2011)

Various factors such as bacterial population, temperature, incubation period, antimicrobial concentration, and the environment around the microbe can influence antimicrobial activity in vitro. Researchers are trying to control factors that can be controlled, for example, choosing the age and condition of the red betel leaves taken, the process of making extractions of red betel leaves (*Piper crocatum* Ruiz and Pav.) using ethanol as a solvent so that chemicals that have an antibacterial effect on the leaves Red betel (*Piper crocatum* Ruiz and Pav.) can be polluted. Then, the bacterial population was standardized by comparing the turbidity with 0.5% Mac Farland solution. The researchers adjusted the temperature, culture media and incubation time according to theory (Kurniawan, 2015).

The antibacterial effectiveness test of red betel leaf (*Piper crocatum* Ruiz and Pav.) extract showed the presence of an inhibition zone which acts as an indicator of the antibacterial effect. It can be concluded that the compounds resulting from the extraction of red betel leaves (*Piper crocatum* Ruiz and Pav.) which diffuse into the agar from the well method are able to inhibit the growth of *Salmonella typhi* bacteria but not *Streptococcus pneumoniae* bacteria. Erythromycin 500 mg was used as a positive control and showed an inhibitory zone for the growth of *Streptococcus pneumoniae* bacteria and Ciprofloxacin 500 mg was used as a positive control for *Salmonella typhi* also showed an inhibitory zone for the growth of the bacteria. Meanwhile, the negative control, namely distilled water, did not show any inhibition zone on the Mueller Hinton Agar media.

In *Salmonella typhi*, the results of the inhibition zone formed prove that the greater the concentration of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract, the greater the antibacterial activity. This is in line with research by Rahmayanti Y in 2014 which stated that the higher the concentration of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract, the more antibacterial active ingredients it contains so that the inhibition zone formed will be larger. Increasing the concentration of antibacterial compounds is expected to increase the penetration of antibacterial compounds into microbial cells.

In this study, there were no inhibitory zone results for red betel leaf (*Piper crocatum* Ruiz & Pav.) extract against *Streptococcus pneumoniae* bacteria. This research is not in line with previous research conducted by Pratiwi I in 2011 which stated that there had been results in the form of a reduction in the number of bacterial colonies by 47.1%. The MIC result of a concentration of 6.25% also indicates that this concentration is active in inhibiting Streptococcus pneumoniae. This may be because the concentration of the extract used must be higher and

the extract is stored for too long so that some of the active antibacterial compounds begin to decrease.

Next, the Kruskal-Wallis test was carried out, this was done to evaluate whether there was a significant difference in the mean number of colonies between groups with variations in extract concentration. There were significant results in the antibacterial activity test of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract against *Salmonella typhi* bacteria. The significance was 0.001 ($p < 0.050$), which means the results were significant (there was a difference in the mean number of colonies in the effect of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract on *Salmonella typhi* bacteria).

Post hoc tests using Mann-Whitney were also carried out again in the study of the inhibitory power of red betel leaf (*Piper crocatum* Ruiz & Pav.) extract against *Salmonella typhi* bacteria and obtained p values <0.05 for most concentrations. This shows that in the post hoc test using Mann-Whitney, a significant difference in the diameter of the inhibition zone was obtained between the control group (positive control and negative control) and the test group (10%, 20%, 30%, 40% and 50% concentration groups). Based on the Mann-Whitney Post hoc test, the positive control group had a significant difference from the 50% concentration group, where the positive control group had a stronger inhibitory effect. The 40% concentration group had a significant difference from the 50% concentration group, where the 50% concentration group had a stronger inhibitory power. The 30% concentration group had a significant difference from the 40% concentration group, and the 40% concentration group had a stronger inhibitory power. The 20% concentration group had a significant difference from the 30% concentration group, where the inhibitory power was stronger in the 30% concentration group. The 10% concentration group had a significant difference from the 20% concentration group, where the 20% concentration group had a stronger inhibitory power.

The positive controls used in this study were Erythromycin on *Streptococcus pneumoniae* bacteria and Ciprofloxacin on *Salmonella typh*i bacteria. From the research results, the positive control had the highest inhibitory effect.

Based on research, the antibacterial substances contained in red betel leaf extract can inhibit the growth of *Salmonella typhi* bacteria, namely saponins, terpenoids, tannins and flavonoids. This is in line with research by Agustina S. Beon Karol Geovani Batista Leki (2016) regarding the analysis of the active compound content contained in red betel leaf (*Piper crocatum* Ruiz & Pav.) extract.

Based on the results of phytochemical tests, red betel leaf extract contains saponins, terpenoids, tannins and flavonoids. Saponins work by denaturing proteins. The surface actives substances of saponin is similar to detergent, therefore saponin can reduce the surface tension of bacterial cell walls and damage membrane permeability in bacteria. Saponin will then diffuse through the cytoplasmic membrane and disrupt the stability of the membrane. Flavonoids work as antibacterials by forming complex compounds with extracellular proteins that have the integrity of bacterial cell membranes. Flavonoids are temporary phenolic compounds that act as protein coagulators (Fadlilah M, 2015)

Terpenoids have antibacterial activity by reacting with porins which are transmembrane proteins in the outer membrane of bacterial cell walls. The terpenoids then form strong polymer bonds and cause damage to the porins. Damage to these porins will reduce the permeability of bacterial cell walls which will result in a number of bacterial cells lacking nutrition. Then the bacteria will experience growth barriers or die (Sudarmi et al., 2017).

The tannin compounds contained in red betel leaf extract also act as antibacterials. The antibacterial mechanism of action of tannins is by lysing bacterial cells. Tannins target bacterial polypeptide walls which cause cell wall formation to be less than perfect and cause bacterial cell death. Apart from that, tannins can also inactivate bacterial enzymes and disrupt the passage of proteins in the inner layers of cells (Sapara et al., 2016).

5 CONCLUSIONS

Red betel leaf (*Piper crocatum* Ruiz & Pav.) extract has inhibitory power against *Salmonella typhi* bacteria but not against *Streptococcus pneumoniae* bacteria. In research conducted on *Salmonella typhi* bacteria, the average inhibition zone formed at a 10% concentration was 0 mm, a 20% concentration was 8.155 mm, a 30% concentration was 8.68 mm, a 30% concentration was 12.295 mm, and the concentration 100% is 14.48 mm.

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