

Inhibitory Power of Papaya Leaves to the Adhesion of Streptococcus Mutans Bacteria to Neutrophils

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Papaya leaves contain several active substances that are thought to inhibit bacterial adhesion. This study aims to determine the inhibitory power of papaya leaf extraction and papaya leaf infusion to the adhesion of Streptococcus mutans bacteria to neutrophils and differences in inhibition in various concentrations. The method used is an in vitro experimental laboratory with a post-test-only control group design. The sample consisted of several concentrations of papaya leaf extraction and papaya leaf infusion (control, 25%, 50%, 75%, and 100%). The treatment group was added with neutrophil isolates and incubated for 3 hours, then exposed to Streptococcus mutans for 2.5 hours. Knowing the adhesion index can be done by calculating the average number of Streptococcus mutans attached to 100 neutrophils. The results showed successively with rounding in control, 25%, 50%, 75%, 100% papaya leaf infusion, adhesion of 9237, 8929, 7436, 3870, 3162 bacteria to neutrophils occurred. The minimum concentration of 25% papaya leaf extraction and 50% papaya leaf infusion can inhibit the adhesion of Streptococcus mutans to neutrophils. There is a difference in the ability between papaya leaf extraction and papaya leaf infusion in inhibiting the adhesion of Streptococcus mutans to neutrophils as follows: papaya leaf extraction 25% to papaya leaf infusion 25% obtained sig value =0.001* with a mean square of -16.38, papaya leaf extraction 50% to Papaya leaf infusion 50% got a sig value of =0.001* with a mean square of -42.91, 75% papaya leaf extraction to 75% papaya leaf infusion got a sig value of =0.001* with a mean square of -28.85 and 100% papaya leaf extraction to 100% papaya leaf infusion obtained a sig value of =0.001* with a mean square of -26.11.

Keywords: Adhesion; Papaya leaf juice; Papaya leaf infusion; neutrophils; Streptococcus mutans.

Microbial normal flora is a group of microorganisms that naturally live on the skin and mucous membranes (mucosa) in normal and healthy humans. Flora in the human body can be permanent or temporary. Normal microbes that persist can not cause disease and may be beneficial in the absence of abnormal conditions. Various things affect the presence of microorganisms in the human body, including nutrition, personal hygiene, living conditions, and changes in diet. One of the

bacteria that is a normal flora microbe but can cause disease is Streptococcus mutans.^{1,2}

Streptococcus mutans is a Gram-positive bacterium that causes dental caries. Dental caries is an infectious disease and is a progressive demineralization process in the hard tissues of the tooth surface from foods containing sugar. Dental caries is the most common disease in the oral cavity, so it is a significant problem for oral health.³

According to the 2007 National Riskesdas Survey in Indonesia, 75% of the Indonesian population had a history of dental caries, with an average number of tooth decay of 5 teeth per person, i.e., 4 of them had been extracted or extracted could no longer be maintained. It is also reported that 23% of Indonesians are aware that they have dental and oral problems. Of those familiar with this, only 30% have received treatment or treatment from dental professionals.⁴

Antibiotics are used to treat infectious diseases. Antibiotics are substances produced by a microorganism, which can inhibit the growth or kill other organisms. However, in developing countries, the emergence of antibiotic-resistant bacterial strains in infectious diseases is a significant problem.⁵

Indonesia is a country rich in natural resources. Indonesia's raw potential is abundant, especially in the agricultural, livestock, fisheries, plantation, forestry, marine sectors, and tourism. The integrated use of natural resources will maximize the existing natural potential to improve the welfare of the Indonesian people. Disease prevention through plants is one of the uses of natural resources in Indonesia.⁶

Indonesia has many types of medicinal plants. The types of plants that are included in the therapeutic plant group reach more than 1000 species. One of them is Papaya (*Carica papaya* L.).⁶ Papaya leaf extract contains antibacterial compounds such as flavonoids, karpain alkaloids, papain enzymes, and tannins.⁷

Lipophilic flavonoids will damage the membrane so that the permeability will increase and interfere with bacterial metabolism. Alkaloids can interfere with the formation of the constituent components of peptidoglycan in bacteria so that the cell wall layer is not fully formed and causes the death of bacteria.⁸ In addition, papain, a proteolytic enzyme, also has a bactericidal and bacteriostatic effect, thus inhibiting the growth of both Gram-positive and Gram-negative bacteria.⁹ The antimicrobial effect of tannins is in the form of inactivating microbial adhesins and inactivating hydrolytic enzymes such as proteases and carbohydrases, as well as inhibiting enzymes in envelope transport proteins.^{10,11}

In a study on the inhibitory power of papaya leaf extract (*Carica papaya* L.) to the

adhesion of bacteria *Porphyromonas gingivalis* to neutrophils, the higher the concentration of papaya leaf extract, the less the adhesion of bacteria to neutrophils.¹² The presence of antibacterial in papaya leaves may inhibit the adhesion of *Streptococcus mutans* to neutrophils because the development of *Streptococcus mutans* is disrupted and dies before adhesion to neutrophils.

People use papaya leaves as traditional medicine by boiling the leaves without extracting them. Currently, there is no research on the juice and infusion of papaya (*Carica Papaya* L.) leaves on the adhesion of *Streptococcus mutans* bacteria to neutrophils. So that researchers are interested in examining the comparison of the inhibition of juice and infusion of papaya leaves (*Carica papaya* L.) to the adhesion of *Streptococcus mutans* bacteria to neutrophils.

METHOD

This research is a laboratory experiment by determining the inhibition of papaya leaf infusion and infusion against the adhesion of *Streptococcus mutans* to neutrophils with concentrations of 25%, 50%, 75%, and 100%. The research was repeated four times. The control was divided into two parts: giving neutrophil cells with 100% infusion without going through incubation and being exposed to *Streptococcus mutans* and 100% infusion control.

The research equipment used was sterile test tubes, ose, volume pipette, micropipette, spirit lamp, Erlenmeyer, incubator, oven, water bath, blender, tube rack, filter paper, centrifuge tube, densitometer, object-glass, coloring rack. Centrifuge, microscope, camera microscope. The research materials consisted of juice and infusion of papaya leaves 100, 75, 50, and 25%, pure strains of *Streptococcus mutans* bacteria, neutrophil isolates, absolute methanol Giemsa dye.

How to Make Papaya Leaf Extract

150 g of papaya leaves that have been washed, dried, and extracted using a juicer into an extract of about 40 mL. The juice specified as a concentration of 100% was accommodated in a sterile Erlenmeyer. Dilution using sterile distilled water to obtain concentrations of 24%, 50%, and 75%.

How to Make Papaya Leaf Infusion

100 g of clean and dry papaya leaves were

cut about 1.5 cm, then put into a sterile 1 L beaker, and 100 mL of sterile distilled water were added. Mixing using a beaker with heating using a water bath for 15 minutes starting at 90 °C while stirring occasionally. Strain the papaya leaf extract using a sterile flannel cloth into a sterile Erlenmeyer glass to obtain an infusion of about 80 mL. Defined as a 100% concentration infused. Infuses with concentrations of 75, 50, and 25% were made using sterile distilled water from 100%.

Treatment for neutrophil cell control without papaya leaf extract or infusion

A total of 100 L of neutrophil isolate with 100 L sterile physiological NaCl was homogenized by gently shaking. Added 200 L of *Streptococcus mutans* isolate by shaking gently. The mixture was incubated at 37 °C incubator for 3 hours, including controls without incubation. The results of the mixture of each treatment made smear preparations. The preparations were fixed using absolute methanol for 5 minutes and stained with Giemsa stain for 30 minutes. The preparations were rinsed with distilled water, then drained. Preparations are ready to be observed under a microscope with a magnifying 1000x.

How to treat bacterial antiadhesion to neutrophil cells from papaya leaf extract

A total of 100 L of papaya leaf juice, each concentration of 25%, 50%, 75%, and 100% with 100 L of the neutrophil isolate, was homogenized by gently shaking. The mixture was incubated at 37 °C incubator for 2.5 hours. Each treatment was exposed to/added bacterial isolates. *Streptococcus mutans* as much as 200 L, homogenized by shaking slowly. The mixture was incubated at 37 °C incubator for 3 hours. The results of the mixture from each treatment were made smear preparations. The preparations were fixed using absolute methanol for 5 minutes. The preparations were stained with Giemsa dye for 30 minutes, rinsed with distilled water, and then drained. Preparations are ready to be observed under a microscope with a magnifying 1000x.

How to treat bacterial antiadhesion to neutrophil cells from papaya leaf infusion

A total of 100 L of papaya leaf infusion, each with a concentration of 25%, 50%, 75%, and 100% with 100 L of neutrophil isolates, were homogenized by gently shaking. The mixture was incubated at 37 °C incubator for 2.5 hours. Each

treatment added 200 U_I of *Streptococcus mutans* isolate by shaking gently. Then the mixture was incubated at 37 °C for 3 hours. The results of the mixture of each treatment made smear preparations. The preparations were fixed with absolute methanol for 5 minutes. The preparations were stained with Giemsa dye for 30 minutes, rinsed with distilled water, and then drained. Preparations are ready to be observed under a microscope with 1000x magnification.

Data analysis

Data processing to test the strength of anti-adhesion between concentrations between juice and infusion using the Anova statistical test to distinguish the strength between juice and infusion followed by multiple comparisons analysis (MCA) with Bonferroni.

RESULTS AND DISCUSSION

Treat With Papaya Leaf Extract

Treatment of control and papaya leaf extract with concentration variations of 25%, 50%, 75%, and 100%, which were contacted with neutrophil cells before being exposed to *Streptococcus mutans* showed differences in the inhibition of adhesion to neutrophils at each concentration (figure1), carried out using the ANOVA statistical test followed by the multiple comparisons test, Bonferroni.

The treatment used as control (V) was between neutrophil cells exposed to *Streptococcus mutans*, bacterial adhesion to neutrophils occurred, as seen from the number of bacteria attached to each neutrophil cell counted in 100 neutrophils with four repetitions obtained the average number of bacteria per 100 cells of 9124.50 with an average value of the number of bacteria per neutrophil cell of 91,245.

Treatment of papaya leaf juice with a concentration of 25%, which contacted neutrophil cells before being exposed to *Streptococcus mutans* inhibited adhesion to neutrophils. It can be seen from the number of bacteria attached to neutrophil cells from each infusion concentration and repeated four times to get the average number of bacteria per 100 cells of 7344.50 with an average value of the number of bacteria per neutrophil cell of 73,445.

Treatment of papaya leaf juice with a concentration of 50%, which contacted

with neutrophil cells before being exposed by Streptococcus mutans inhibited adhesion to neutrophils, it was seen from the number of bacteria attached to neutrophil cells from each concentration of infusion and repeated four times the average value was obtained bacteria per 100 cells is 3191.00 with an average value of the number of bacteria per neutrophil cell is 31.910.

Treatment of papaya leaf juice with a concentration of 75%, which contacted neutrophil cells before being exposed to Streptococcus mutans, inhibited adhesion to neutrophils. it was seen from the number of bacteria attached to neutrophil cells from each concentration of infusion and repeated four times to get the average value

of bacteria per 100 cells is 973.50 with an average value of the number of bacteria per neutrophil cell is 9.735.

Treatment of papaya leaf juice with a concentration of 100%, which contacted neutrophil cells before being exposed to Streptococcus mutans inhibited adhesion to neutrophils. It was seen from the number of bacteria attached to neutrophil cells from each concentration of infusion and repeated four times to get the average value of bacteria per 100 cells is 546.00 with an average value of the number of bacteria per neutrophil cell is 5,460.

Papaya leaf infusion treatment

Control and treatment with papaya leaf infusion with concentration variations of

Table 1. The number of Streptococcus mutans in neutrophils in contact with papaya leaf juice

Group	Mean (Neutrophil)	CI 95%		*P
		min	max	
P-I (25%)	7344.50	7345	7377	≥ 0.001*
P-II (50%)	3191.00	3185	3278	
P-III (75%)	973.50	962	982	
P-IV (100%)	546.00	531	562	
V (control)	9124.50	8993	9374	

*One-way ANOVA, α = 5%.

*Significant (p ≤ 0,05).

Post hoc Bonferroni: control vs 25%: ≥ 0.001 ;25% vs 50%: ≥ 0.001 ; 25% vs 75%: ≥ 0.001 ; 25% vs 100%: ≥ 0.001 ; control vs 50%: ≥ 0.001 50% vs 75%: ≥ 0.001; 50% vs 100%: ≥ 0.001; control vs 75%: ≥ 0.001; 75% vs 100%: 1.00; control vs 100%: ≥ 0.001

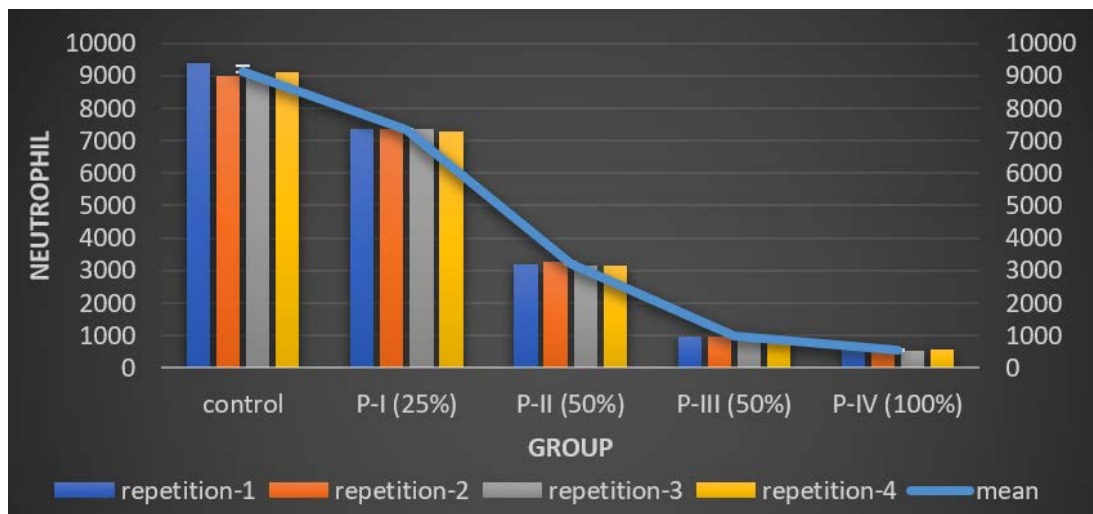


Fig. 1. The number of Streptococcus mutans in neutrophils in contact with papaya leaf juice

25%, 50%, 75%, and 100%, which contacted with neutrophil cells before being exposed to *Streptococcus mutans*, showed differences in inhibition of adhesion to neutrophils at each concentration, as seen from the number of bacteria attached, on neutrophil cells (figure 2). The confirmation test was carried out using the ANOVA statistical test followed by the multiple comparisons test, Bonferroni.

The treatment used as a control was V between neutrophil cells exposed to *Streptococcus mutans*, bacterial adhesion to neutrophils occurred, as seen from the number of bacteria attached to each neutrophil cell counted in 100 neutrophils with four repetitions obtained the average number of bacteria per 100 cells with an average value of

9236.50, the number of bacteria per neutrophil cell is 92.365.

Treatment of papaya leaf infusion with a concentration of 25%, which contacted with neutrophil cells before being exposed to *Streptococcus mutans*, inhibited adhesion to neutrophils. it was seen from the number of bacteria attached to neutrophil cells from each concentration of infusion and repeated four times to get the average value of bacteria per 100 cells is 8928.50, with an average value of the number of bacteria per neutrophil cell is 89.285.

Treatment of papaya leaf infusion with a concentration of 50%, which contacted with neutrophil cells before being exposed to *Streptococcus mutans* inhibited adhesion to

Table 2. *Streptococcus mutans* counts in neutrophils contact with a papaya leaf infusion

Group	Mean (Neutrophil)	CI 95% min	^a P max	
I – I (25%)	8928.50	8871	9061	≥ 0.001*
I-II (50%)	7435.75	7362	7509	
I-III (75%)	3870.00	3839	3909	
I-IV (100%)	3162.00	3145	3172	
V (kontrol)	9236.50	8983	9374	

^aOne-way ANOVA, α = 5%.

*Significant (p ≤ 0,05).

Post hoc Bonferroni: control vs 25%: ≥ 1.00 ;25% vs 50%: ≥ 0.001 ; 25% vs 75%: ≥ 0.001 ; 25% vs 100%: ≥ 0.001 ; control vs 50%: ≥ 0.001 50% vs 75%: ≥ 0.001; 50% vs 100%: ≥ 0.001; control vs 75%: ≥ 0.001; 75% vs 100%: 0.077; control vs 100%: ≥ 0.001

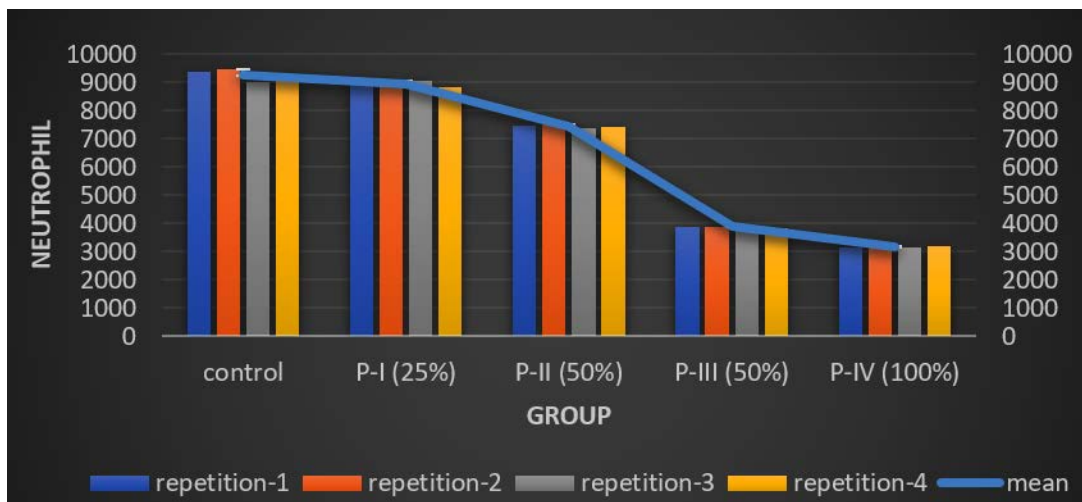


Fig. 2. *Streptococcus mutans* counts in neutrophils contact with a papaya leaf infusion

neutrophils. It can be seen from the number of bacteria attached to neutrophil cells from each infusion concentration and repeated four times to get the average number of bacteria per 100 cells of 7435.75 with an average value of the number of bacteria per neutrophil cell of 74.358.

Treatment of papaya leaf infusion with a concentration of 75%, which contacted with neutrophil cells before being exposed to *Streptococcus mutans* inhibited adhesion to neutrophils. It can be seen from the number of bacteria attached to neutrophil cells from each concentration of infusion and repeated four times to get the average number of bacteria per 100 cells of 3870.00 with an average value of the number of bacteria per neutrophil cell of 38.700.

Treatment of papaya leaf infusion with a concentration of 75%, which contacted with neutrophil cells before being exposed to *Streptococcus mutans*, inhibited adhesion to neutrophils. It can be seen from the number of bacteria attached to neutrophil cells from each concentration of infusion and repeated four times to get the average number of bacteria per 100 cells of 3870.00 with an average value of the number of bacteria per neutrophil cell of 38.700.

There is antiadhesion activity of papaya leaf extract starting from a concentration of 25%. Anti adhesion activity in papaya leaf infusion starting from a concentration of 50% because the content of antibacterial ingredients from papaya leaf extract is more because the section does not experience dilution. In contrast, infusion there is the addition of water.

The higher the concentration of papaya leaf juice or infusion, the stronger the anti-adhesion power against the test bacteria; this is evidenced by the difference in the mean square value with the higher the concentration of the anti-bacterial juice, the higher the mean square value, the higher the adhesion inhibition. As a result, bacterial adhesion or bacterial attachment to neutrophils decreases.

The higher the concentration of papaya leaf infusion, the stronger the antiadhesion power against the test bacteria; this is evidenced by the difference in the mean square value with the higher the concentration of juice, the higher the mean square value except for a concentration of 25%, the value of sig 1.000 even though numerically there is

a difference with the mean square value of -3.91.

There is a difference in the ability of papaya leaf juice (papaya leaf juice) and papaya leaf infusion (papaya leaf infusion) in inhibiting the adhesion of *Streptococcus mutans* to neutrophils as follows: papaya leaf juice 25% to papaya leaf infusion 25% obtained sig value $e^{*0.001}$ with mean square - 16.38, 50% papaya leaf infusion to 50% papaya leaf infusion received a sig value of $e^{*0.001}$ with a mean square of -42.91, 75% papaya leaf juice to 75% papaya leaf infusion received a sig value of $e^{*0.001}$ with a mean square of -28.85 and 100% papaya leaf juice to 100% papaya leaf infusion received a sig value of $e^{*0.001}$ with a mean square of -26.11.

CONCLUSIONS

There is the ability of papaya leaf juice to inhibit the adhesion of *Streptococcus mutans* to neutrophils with a sig value of $e^{*0.001}$ from 25%. The minimal concentration of papaya leaf juice can inhibit the adhesion of *Streptococcus mutans* to neutrophils at a concentration of 25%. There is the ability of papaya leaf infusion to inhibit the adhesion of *Streptococcus mutans* to neutrophils starting from a concentration of 50%. The minimal concentration of papaya leaf infusion can inhibit the adhesion of *Streptococcus mutans* to neutrophils at a concentration of 50%.

Based on the results obtained, it is recommended to conduct further research on the adhesion inhibition of *Streptococcus mutans* bacteria with variations in contact time between papaya leaf infusion and infusion to neutrophil cells and determine the minimum inhibitory concentration of papaya leaf infusion and infusion against antiadhesion to neutrophil cells.

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Conflicts of Interest

There are no conflicts of interest.

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