Effectiveness Of Tamarind Leaf (Tamarindus Indica L.) Ethanol Extract Antibacterial Against Porphyromonas Gingivalis

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Submission date: 08-Dec-2023 08:48AM (UTC+0700)
Submission ID: 2251966531
File name: 16089-46103-1-SM.pdf (252.13K)
Word count: 4572
Character count: 24340

DENTINO
JURNAL KEDOKTERAN GIGI
Vol VIII. No 1. March 2023

EFFECTIVENESS OF TAMARIND LEAF (*Tamarindus indica* L.) ETHANOL EXTRACT ANTIBACTERIAL AGAINST *Porphyromonas gingivalis*

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ABSTRACT
Background: One of the initial measures to prevent chronic periodontitis is the use of mouthwash. Chlorhexidine is an antibacterial agent that can be used as a mouthwash. Chlorhexidine has side effects so that a new method is needed to prevent periodontitis with fewer side effects, namely by using plants as a medicine, one of the plants containing active compounds with antibacterial effects. The ethanol extract of tamarind leaf is proven to contain active compounds of flavonoids, tannins, alkaloids, and saponins. **Purpose:** The aim of the study was to analyze the antibacterial effect of ethanol extract of tamarind leaf (*Tamarindus indica* L.) leaf against *Porphyromonas gingivalis* bacteria. **Material and Methods:** The research is an experimental laboratory type using the disc diffusion method (Kirby-Bauer), namely the paper disc diffusion method with the test material of ethanol extract of tamarind leaf (*Tamarindus indica* L.) in various concentrations, namely: 3.125%, 6.25%, 12.5 %, 25%, 50%, and 100%. **Results:** Based on the results of the research on the antibacterial effect of the ethanolic extract of tamarind leaf with concentrations of 3.125%, 6.25%, and 12.5% with an average diameter of 0.00 mm of inhibition zone. At concentrations of 25%, 50%, and 100%, they fall into the medium criteria group, because they have an average inhibition zone diameter of 5.21 mm, 7.45, and 9.16 mm, so that the concentration of 100% is closest to the average the mean diameter of the positive control group or chlorhexidine. **Conclusion:** It can be concluded that the ethanol extract of tamarind leaf (*Tamarindus indica* L.) has an antibacterial effect that can inhibit the growth of *Porphyromonas gingivalis* bacteria at a concentration of 100% with an average inhibition zone diameter of 9.16 mm.

***Keywords*:** Antibacterial, *Porphyromonas gingivalis*, Tamarind leaf
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INTRODUCTION

Periodontal disease is a pathological condition that causes inflammation and damage to the supporting tissue, namely cementum, gingiva, periodontal ligament, and alveolar bone.[1] Periodontal disease is the second public dental health problem after caries, it is reported that the prevalence of periodontal disease in Indonesia reaches 74.1%.[2,3] The report above adequately illustrates the high risk of the Indonesian people experiencing periodontal disease.[4] According to *American Academy of Periodontoly* (AAP) periodontal disease is classified into chronic periodontitis and aggressive periodontitis.[4,5] One of the most common periodontal diseases in adulthood is chronic periodontitis.[4]

Chronic periodontitis is a state of inflammation of the supporting tissue of teeth consisting of gingiva, periodontal ligament, alveolar bone and cementum. This disease is caused by certain bacteria which can damage the supporting tissue of teeth so that the teeth become loose, pockets form, and loss of the gingival attachment.[6,7] Bacteria that are often involved as a cause of chronic periodontitis is *Porphyromonas gingivalis*.[8] Putri and Bachtiar's research (2020) states that bacteria *Porphyromonas gingivalis* found as much as 85.75% in subgingival plaque of patients with chronic periodontitis and chronic periodontitis cases can be found around 40-100% caused by bacteria ini.[9]

Porphyromonas gingivalis bacteria capable to cause pathological changes in the supporting tissue of the teeth then cause inflammation which affects the periodontium cells directly.[10] *Porphyromonas gingivalis* are bacteria that play the role as a cause of periodontitis, the main characteristic of these bacteria

is gram-negative bacteria, *non-motile*, rod shaped, *assacharolytic,* and anaerobic.[9]

The goals of chronic periodontitis treatment are to remove the pathological biofilm and heal the inflammation. Treatment of this case can be done by *scalling, root planning*, and use of antibiotics to inhibit the growth of periodontal pathogens. One of the initial actions to prevent chronic periodontitis is to use mouthwash.[11,12] Chlorhexidine widely used as an antibacterial mouthwash because it is the gold standard that is effective in reducing the growth of bacteria in the oral cavity.[13] However, it can cause several side effects such as resistance. If it used for long term it can causes a yellow to brown color, has a bitter taste, and can cause interference with the tongue or parotid glands.[14,15] Based on the side effects of chlorhexidine above, a new method is needed to prevent periodontitis with fewer side effects, namely using herbal plants.[16]

The use of plants as medicine has long been known, one of the plants that has antibacterial power because of it's biologically active content, namely tamarind leaf (*Tamarindus indica* L.).[17-19] Previous research also reported that ethanol extract of tamarind leaf was proven to have an antibacterial effect against several types of bacteria.[20] The ethanol extract of tamarind leaf is proven to contain active compounds of flavonoids, alkaloids, tannins and saponins.[18,19] Flavonoid and alkaloid compounds are able to inhibit bacterial cell proteins so that they precipitate and stop the metabolic activities of bacterial cells. The ability of tannin compounds to inhibit the formation of cell walls and enzymes in bacteria, as well as saponin compounds that can damage bacterial membrane.[18,20]

Kalirajan's research (2018) on *Escherichia coli* bacteria proved that the ethanol extract of tamarind leaf had an antibacterial effect.[19] Based on the description above, researchers were interested in knowing the antibacterial effectiveness of the ethanol extract of tamarind leaf with concentrations of 3.125%, 6.25%, 12.5% , 25%, 50%, and 100% for *Porphyromonas gingivalis*.

MATERIAL AND METHODS

This study used the paper disc diffusion method with the ethanol extract of tamarind leaf as the test material (*Tamarindus indica* L.) in various concentrations, namely: 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%, negative control with DMSO solution because it has no antibacterial activity and positive control with chlorhexidine solution. The sample of this research is *Porphyromonas gingivalis* ATCC 33277.

Tamarind leaf were obtained from the Manoko Experimental Garden, Lembang, district. West Bandung, Bandung, which was then carried out by plant determination at the Directorate of Scientific Collections Management of BRIN, Cibinong with plant identification number B-3/II.6.2/DI.05.07/6/2022. Phytochemical tests were carried out at the Central Laboratory of Padjadjaran University, Bandung with Analysis No. S-392/LS-BA.36/2022.

The ethanol extract of tamarind leaf was prepared by washing 8 kg of tamarind leaf, then drying them for 2x24 hours in an oven. Grind the dried tamarind leaf to obtain simplicia. Put the simplicia into the maceration vessel, along with 96% ethanol for maceration. Place the maceration vessel in a dark place so that it is protected from air, light or moisture, and strain the simplicia. The liquid simplicia that had been filtered was evaporated using a rotary evaporator at 40°C, until a thick extract was obtained for 3 hours to obtain the ethanol extract of tamarind leaf. 21 After the extract became thick to as much as 500 ml, the extract was diluted using DMSO to obtain a concentration of 0% as a control. negative, 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%.

Dilution of Tamarind Leaf Extract stock (EDAJ) was carried out using 10% DMSO to make concentration series. The series of extract concentrations used are as follows:

EDAJ 100% : Stock solution (1 mg extract + 1 mL DMSO 100%)
EDAJ 50% : 500 μL stock solution + 500 μL DMSO 10% (Solution A)
EDAJ 25% : 500 μL solution A + 500 μL DMSO 10% (Solution B)
EDAJ 12.5% : 500 μL solution B + 500 μL DMSO 10% (Solution C)
EDAJ 6.25% : 500 μL solution C + 500 μL DMSO 10% (Solution D)
EDAJ 3.125% : 500 μL solution D + 500 μL DMSO 10%

Making Growing Media *Porphyromonas gingivalis*

The process for making *Porphyromonas gingivalis* growing media is as follows:

MHA medium was prepared by dissolving 19 grams of MHA medium in 500 mL ddH_2O, while MHB medium was prepared by dissolving 10.5 grams of MHB medium in 500 mL ddH_2O. The medium was heated using a microwave and homogenized and sterilized using an autoclave at 121°C with a pressure of 1.5 atm for 20 minutes. Then the MHA medium was poured into a petri dish to make agar plates.

The working procedure for the disk diffusion method consists of preparing a bacterial inoculum. Inoculation of Porphyromonas gingivalis colonies on MHA into MHB medium. Using a homogenized suspension vortex mixer, then the turbidity of the solution was adjusted to 0.5 McFarland turbidity with the aim of obtaining an inoculum with a bacterial count range of 1-2 × 10^8 CFU/mL.

Disk Diffusion Agar can be done by dipping a sterile cotton swab into the bacterial suspension, then

pressing the cotton swab against the wall so that there is no excess suspension. Apply the cotton swab evenly to the surface of the MHA, leave it for 3-5 minutes until the suspension is absorbed. Place a paper disc (6 mm) on the agar plate and drop 20 μL of ethanol extract of tamarind leaf of various concentrations, negative control and positive control and let stand until the solution is completely absorbed. Each treatment was repeated 4 times and incubated at 37^{o}C for 24 hours.

The diameter of the inhibition zone was measured, namely the zone where no growth or formation of *Porphyromonas gingivalis* bacteria was found on the discs using a vernier caliper. According to Davis-Stout, the criteria for antibacterial strength, namely the diameter of the inhibition zone, are divided into several categories, namely weak (≤ 5 mm); Medium (5-10 mm); Strong (10-20 mm); and very strong (> 20 mm).[22]

The data assessed was the diameter of the growth inhibition zone of *Porphyromonas gingivalis* bacteria in mm units that had been treated with ethanol extract of tamarind leaf (*Tamarindus indica* L.) in vitro. This study used the Kirby-Bauer disc diffusion method to determine the diameter of the inhibition zone. The normality test was carried out followed by a homogeneity test based on the Kruskal-Wallis test. Then the Mann Whitney test was carried out to determine whether there was a significant difference in the level of antibacterial effectiveness against Porphyromonas gingivalis. Statistical tests were carried out using the IBM Statistics SPSS 22 application.

RESULT

The results of the tamarind leaf phytochemical screening test can be seen in Table 1.

Table 1. Results of Phytochemical Screening Test of Tamarind Leaf Extract

No	Metabolites Secondary	Test Method	Result
1	Tanin	Reactor $FeCl_3$ 1%	+
2	Flavonoid	a. Reactor HCl pekat + Mg	-
		b. Reactor H_2SO_4 2N	-
		c. Reactor NaOH 10%	+
3	Saponin	Heated	-
4	Alkaloid	Reactor Dragendorff	+

Information:
+ : Detected
- : Undetected

This research was carried out by giving 8 treatments of tamarind leaf ethanol extract on bacteria *Porphyromonas gingivalis*. There were 6 concentrations of tamarind leaf ethanol extract given, namely: 3.125%, 6.25%, 12.5%, 25%, 50%, 100%. Subsequent treatment was a positive control with 0.2% chlorhexidine solution and a negative control with distilled water to observe the antibacterial effect produced by the ethanol extract of tamarind leaf *in vitro*.

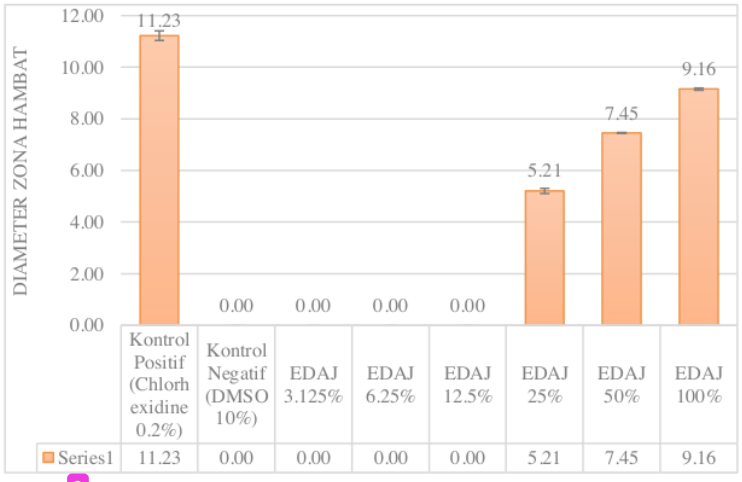

Chart 1. The diameter of the inhibition zone of tamarind leaf extract against *Porphyromonas gingivalis*

Based on Table 2 and Chart 1, this study yielded an average diameter of the inhibition zone of tamarind leaf extract in the positive control group of 11.23 mm, the average inhibition zone in the 100% tamarind leaf extract group was 9.16 mm, the extract group 50% tamarind leaf extract by 7.45 mm, 25% tamarind leaf extract group by 5.21 mm, 12.5% tamarind leaf extract group by 0.00 mm, 6.25% tamarind leaf extract group by 0 .00 mm and in the 3.125% tamarind leaf extract group by 0.00 mm. Based on these results it showed that the 100% tamarind leaf extract group was closest to the average diameter of the positive control or chlorhexidine group. Based on Table 1 there is a standard deviation (*stdev*) or the size of the deviation of the data from the average results obtained, the smaller the value the better the level of accuracy of data analysis, the smaller the standard deviation (closer to 0) indicates the data is more homogeneous, while the larger the standard deviation indicates heterogeneous data.

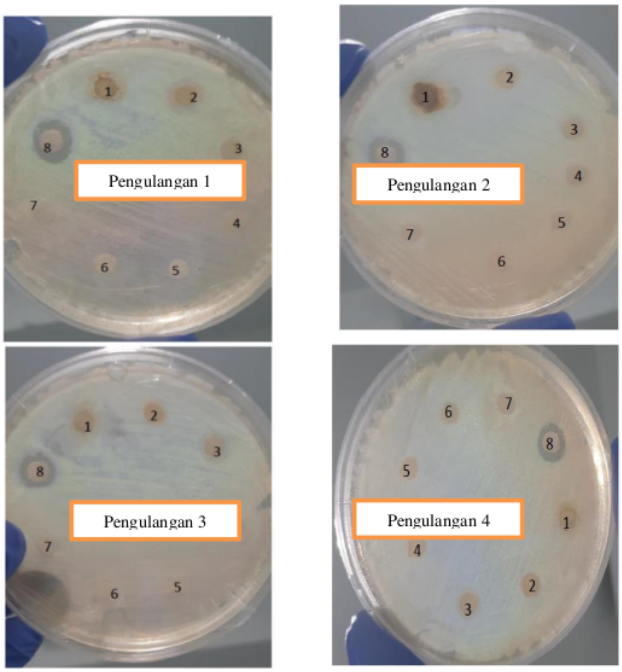

Figure 1. Observation of the inhibition zone of tamarind leaf extract against *Porphyromonas gingivalis* (repetition 1, 2, 3, and 4) Information: (1) Tamarind Leaf Extract 100%; (2) Tamarind Leaf Extract 50%; (3) Tamarind Leaf Extract 25%; (4) Tamarind Leaf Extract 12.5%; (5) Tamarind Leaf Extract 6.25%; (6) Tamarind Leaf Extract 3.125%; (7) Negative Control (DMSO 10%); (8) Positive Control (0.2% Chlorhexidine).

This study conducted a normality test before analyzing the comparison of each group. The normality test aims to determine whether the data obtained for the comparative analysis is normally distributed or not. The data is said to be normally distributed if the significance value is ≥ 0.05. The normality test uses the Shapiro-Wilk test, which is a method that can be used for data with a sample size below 50. Based on the results of the normality test at a concentration of 25%, 50%, and 100% it has a significance value of ≥ 0.05, but at a concentration of 3.125 %, 6.25%, and 12.% obtained a value of 0.000, meaning that the data is not normally distributed or the significance is ≤ 0.05. Because there are data that are not normally distributed, the One-Way ANOVA test cannot be continued, considering that the requirements for the One-Way ANOVA test are that the data must be normally distributed.

Table 2. Comparison test results *Kruskall-Wallis*

Test Statistics[a,b]

	Concentration
Chi-Squre	30,740
df	7
Asymp. Sig.	**0,000**

a. *Kruskall-Wallis Test*
b. *Grouping Variabel :* inhibition zone

Homogeneity test is a test used to determine whether the data used is homogeneous or not. Data that is not normally distributed will be analyzed using non-

parametric statistics, namely the Kruskall-Wallis test. The Kruskall-Wallis test is an alternative that can be used if the One-Way ANOVA test is not normally distributed or is not homogeneous. Based on Table 2, the Kruskall-Wallis test yields a value of 0.000 or a significance of ≤ 0.05 so that H1 is accepted and H0 is rejected.

This study conducted the Mann Whitney Non-Parametric Post Hoc test to determine the comparison of sample groups, the Mann Whitney Non-Parametric Post Hoc test can be used if the data does not meet the assumption of normality. Based on the results of the Mann Whitney Non-Parametric Post Hoc test it yielded a significance value of ≤ 0.05 which indicated that there was a significant difference, meaning that H1 was accepted, so that it can be concluded that from each concentration group tested there was a significant difference, because the value was ≤ 0.05.

Table 3. Test results of *Post Hoc Non-Parametric Mann Whitney*

Perlakuan	KP	KN	EDAJ 3.125%	EDAJ 6.25%	EDAJ 12.5%	EDAJ 25%	EDAJ 50%	EDAJ 100%
KP		0.014*	0.005*	0.014*	0.014*	0.021*	0.021*	0.021*
KN			1TB	1TB	1TB	0.014*	0.014*	0.014*
EDAJ 3.125%				1TB	1TB	0.014*	0.014*	0.014*
EDAJ 6.25%					1TB	0.014*	0.014*	0.014*
EDAJ 12.5%						0.014*	0.014*	0.014*
EDAJ 25%							0.014*	0.014*
EDAJ 50%								0.021*
EDAJ 100%								0.021*

Ket :
*: Bermakna
TB : Tidak Bermakna

This shows that between groups concentration of ethanol extract of tamarind leaf has significantly different effectiveness, the higher the concentration, the higher the inhibition in inhibiting bacterial growth *Porphyromonas gingivalis*.

DISCUSSION

The criteria used to classify control inhibition zones and sample materials in this study were according to Davis-Stout. Based on Davis-Stout, the inhibition zone with a weak category has a diameter of 5 mm or less, the medium category has an inhibition zone diameter of 5-10 mm, the strong category has an inhibition zone diameter of 10-20 mm, the very strong category has an inhibition zone diameter of 20 mm or so.[21] The results showed that the negative control group and the ethanol extract of tamarind leaf at concentrations of 3.125%, 6.25%, 12.5% belonged to the weak category, while the positive control group belonged to the strong category, and the ethanol extract of tamarind leaf to the weak category. concentrations of 25%, 50%, 100% belong to the medium category. In this study, a normality test was carried out using the Shapiro-Wilk test and the results obtained at concentrations of 25%, 50%, and 100% had a significance value of ≥ 0.05, which means that the data were normally distributed, but at concentrations of 3.125%, 6.25%, and 12.% obtained a value of 0.000 meaning that the data is not normally distributed or a significance ≤ 0.05. Furthermore, the data will be analyzed with non-parametric statistics, namely the Kruskall-Wallis test and produce a value of 0.000 or a significance ≤ 0.05.

The data was then tested Post Hoc with Non-Parametric Mann Whitney and yielded a significance value of ≤ 0.05, so there was a significant difference between the two groups and it can be concluded that for each concentration group tested there was a significant difference because the value was ≤ 0.05. This shows that between groups concentrations of ethanol extract tamarind leaf have significantly different effectiveness, namely the higher the concentration, the higher inhibitory power in inhibiting *Porphyromonas gingivalis* bacterial growth. This can happen because the higher the concentration of ethanol extract tamarind leaf, the higher content of active compounds that have an antibacterial role such as tannins, flavonoids, and alkaloids. The ethanol extract of tamarind leaf has an anti-bacterial effect on *Porphyromonas gingivalis*, however the results were not as strong as the antibacterial effect produced by the positive control. The antibacterial effect produced by Chlorhexidine 0.2% as a positive control is higher because Chlorhexidine is the gold standard that has been widely used in dentistry in killing gram positive or negative bacteria.[23]

Chlorhexidine has a positive charge that can be strongly attracted to the bacterial cell wall, which then causes a leak in the cell membrane resulting in a change in the integrity of the bacterial cell membrane and can chemically damage the cytoplasm.[24]

In accordance with research conducted by Norkholisoh (2018) stated that the ethanol extract of tamarind leaf contained active compounds saponins, tannins, flavonoids, and alkaloids, but in the results of the phytochemical screening test on the ethanol extract samples of tamarind leaf used for this study, the samples contained active compounds. tannins, flavonoids, and alkaloids, and no saponins were detected.[19] The content of active saponin compounds is not detected due to the drying process of the leaf before being made into extracts, active saponin compounds are susceptible to high temperatures, so these active compounds can be damaged when heated to very high temperatures, the content of active saponin compounds is not detected can also occur due to processing. Extraction is too fast so that the active compounds of saponins from tamarind leaf are not extracted properly.[25]

The mechanism of action from active tannin compound as an antibacterial agent against *Porphyromonas gingivalis* bacteria is by inhibiting the production of enzymes in the inner layer of cells because the tannin active compound has a target on the bacterial cell wall polypeptide which will disrupt the

process of forming bacterial cell wall. This causes the bacterial cell wall not to form completely.[26] The formation of an imperfect cell wall results in leakage of bacterial cells and facilitates the entry of antibacterial compounds. Antibacterial compounds will damage enzyme activity by inhibiting enzyme production in cells which causes bacterial death.[26] Tannins can also interfere with cell wall permeability and cause wall damage due to cell wall shrinkage.[27]

The mechanism of action from active compounds of flavonoids as antibacterial agents is by destroying the permeability of the bacterial walls, microsomes, and lysosomes of *Porphyromonas gingivalis*.[26] This happens because the active compounds of flavonoids can inhibit bacterial metabolism by denaturing proteins and damaging cell membranes.[26,28] Damage to the cell membrane will disrupt the energy formation process by inhibiting the use of oxygen by bacteria and result in bacterial energy metabolism stopping and bacterial lysis.[28,29]

Mechanism of action from active alkaloid compounds as antibacterial agents against bacteria *Porphyromonas gingivalis*, by inhibiting the formation of the bacterial cell wall.[26] This causes a change in the permeability of the cell membrane which will facilitate the entry of antibacterial compounds into the cell and the release of cell substances such as nucleic acids and bacterial cell proteins, causing the death of the bacterial cell.[30]

Based on the results of the study it was found that between groups concentration of ethanol extract tamarind leaf had significantly different effectiveness, namely the higher the concentration, the higher the inhibitory power in inhibiting bacterial growth *Porphyromonas gingivalis*. However, this ability cannot be compared with the positive control, namely 0.2% chlorhexidine which is the gold standard used in dentistry.Based on the results of the study, it was concluded that the ethanol extract of tamarind leaf had effectiveness as an antibacterial against *Porphyromonas gingivalis* at concentrations of 25%, 50% and 100%.

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