

Antibacterial activity of longan seed extract (*Dimocarpus longan L.*) to *P.gingivalis*

Aktivitas antibakteri ekstrak biji kelengkeng (*Dimocarpus longan L.*) terhadap *P.gingivalis*

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ABSTRACT

Longan (*Dimocarpus longan L.*) is a plant that has potential as an antibacterial. Longan seeds are part of the longan plant which contains antibacterial compounds in the form of phenolics, tannins, flavonoids, and triterpenoids. This research is aimed to determine the antibacterial effect of longan seeds on the bacterium *Porphyromonas gingivalis* ATCC 332277 which causes chronic periodontitis by calculating the average diameter of the inhibition zone. This research was conducted using experimental laboratory methods with groups at concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, 75%, 100%. In comparison, chlorhexidine 0.2% as a positive control and DMSO 10% as a negative control. Based on the well diffusion method, the results showed that in the experimental group, a concentration of 100% resulted in a 10.07 mm zone of inhibition but not greater than chlorhexidine 0.2% which has 11.94 mm. At the same time, the smallest inhibition zone was produced by longan seeds with 25% extract which was 3.15 mm. It is concluded that there is an effect of longan seed extract on inhibiting the growth of *P.gingivalis* bacteria.

Keyword: chronic periodontitis, longan seed extract, *P.gingivalis*, agar well diffusion method, antibacterial

ABSTRAK

Kelengkeng (*Dimocarpus longan L.*) merupakan salah satu tanaman yang berpotensi sebagai antibakteri. Biji merupakan bagian dari tanaman kelengkeng yang mengandung senyawa antibakteri berupa fenolat, tanin, flavonoid, dan triterpenoid. Artikel ini membahas efek antibakteri biji kelengkeng terhadap bakteri *Porphyromonas gingivalis* ATCC 332277 penyebab periodontitis kronis dengan menghitung rata-rata diameter zona hambat. Penelitian ini dilakukan dengan metode eksperimen laboratorik dengan kelompok eksperimen pada konsentrasi 3,125%, 6,25%, 12,5%, 25%, 50%, 75%, 100%. Sebagai pembeda, klorheksidin 0,2% sebagai kontrol positif dan DMSO 10% sebagai kontrol negatif. Berdasarkan metode difusi isumur, tampak bahwa pada kelompok eksperimen, konsentrasi 100% menghasilkan zona hambat 10,07 mm namun tidak lebih besar dari klorheksidin 0,2% sebesar 11,94 mm. Sedangkan zona hambat terkecil dihasilkan oleh biji kelengkeng dengan ekstrak 25% yaitu 3,15 mm. Disimpulkan bahwa terdapat pengaruh ekstrak biji kelengkeng dalam menghambat pertumbuhan bakteri *P.gingivalis*.

Kata kunci: periodontitis kronis, ekstrak biji kelengkeng, *P.gingivalis*, metode difusi isumur agar, antibakteri.

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INTRODUCTION

Periodontitis is a chronic inflammatory disease related to plaque biofilm dysbiosis marked by the progressive destruction of teeth-supporting tissues. Periodontitis can cause tooth loss if left untreated, though most cases can be prevented and treated.¹ The data from the *Riset Kesehatan Dasar* or Baseline Health Research in 2018 states a high prevalence of periodontitis in Indonesia, which reached 74.1%.² Chronic periodontitis is also known as *adult periodontitis* or *chronic adult periodontitis* because it is often found in adults. This is the most common form of periodontitis and is considered slow progressing by destroying the supportive tissues surrounding the teeth.³

Periodontitis is characterized by forming a periodontal pocket, gingival inflammation, loss of periodontal attachment, vertical and horizontal loss of alveolar bone, and tooth mobility. Although a slow-progressing disease, the progressive lesion tends to develop more rapidly in the interproximal region, which has high plaque accumulation and areas difficult to reach by plaque control measures, such as furcation, overhanging margin, and

malposition teeth.⁴ Chronic periodontitis occurs because of the involvement of anaerobic bacteria. Several pathogens found in chronic periodontitis include *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythensis*, *Fusobacterium nucleatum*, *Treponema*, *Campylobacter rectus*, *Eikenella corrodens*, *Peptostreptococcus micros*, and *Eubacterium species*.⁵

P.gingivalis are non-motile, rod-shaped, black-pigmented, anaerobic pathogenic gram-negative bacteria that colonize in periodontal pockets. These bacteria are included in the phylum *Bacteroidetes*.^{6,7} Based on 42 studies from 1993-2016 using the meta-analysis method, 34% of *P.gingivalis* were found in healthy individuals and 78% in individuals with periodontal disease.⁸ This encourages the use of herbal ingredients as an alternative treatment of periodontitis. A study proves the presence of active compounds such as flavonoids found in plants that can provide an antibacterial effect, one of the plants that have these compounds is longan (*Dimocarpus Longan L.*).⁹ Apart from the flesh of the fruit consumed by the wide community, longan seeds account for

a total of 17% of the total weight of longan fruit which becomes waste or is burned as biomass fuel.¹⁰ Longan seeds can be used as a natural treatment because of the presence of bioactive compounds such as phenolics, flavonoids, tannins, saponins, and polysaccharides.^{11,12}

The use of chlorhexidine mouthwash is recommended in the treatment of periodontitis. Chlorhexidine is an antiseptic with wide-spectrum antibacterial and antifungal effects, which has been proven effective in treating periodontal diseases, including periodontitis.¹³ The concentration of chlorhexidine in over-the-counter mouthwash is low, from 0.1%, 0.12%, 0.2%, to 0.5%.¹⁴ Chlorhexidine can cause side effects of xerostomia, hypogesia, tooth discoloration, and coated tongue.¹³

This study evaluate the effect of antibacterial activity of longan seed extract to *P.gingivalis*.

METHODS

Collection of simplicia and materials

This study used the seeds of longan obtained from a community plantation in Cijambe District, Subang, West Java which the Microbiology Laboratory of Padjadjaran University Bandung had determined. The making of longan seed extract was carried out using the maceration method and was dissolved in 96% ethanol.

Making of longan seed extract

Four kilograms of longan were rinsed clean with running water. After the cleaning process, the seeds were separated from the skin and fruit, then ground and dried under indirect sunlight. They were mashed with a blender, sifted, and scaled at 2 kg. The longan seeds that have been refined were inserted into a macerator and dissolved in 96% ethanol. The seeds were immersed for five days for 24 hours while stirred occasionally. The immersion process was conducted in a dark chamber to prevent sample reaction to the sun. After the immersion process, the dregs and filtrate were filtered with filter paper, then the solvent was evaporated with a rotary evaporator to obtain a concentrated longan seed extract. Longan seed extracts were diluted with 10% DMSO with concentrations, i.e., 3.125%, 6.25%, 12.5%, 25%, 50%, 75%, and 100%.

Phytochemical screening

Alkaloid was tested by adding 4 mL of longan seed extract and 2 mL of chloroform added by 5 mL of 10% ammoniac (NH3) and then 10 drops of concentrated H₂SO₄ was added into the test tube. For the flavonoid was tested by adding 1 mL of longan seed extract and 10% NaOH. Saponin was test by adding 10 mL of longan seed extract were shaken vertically for 10 seconds and kept still for 10 seconds. Tannin was tested by adding 1 mL of longan seed extract was taken and inserted into a reaction tube, then mixed with 2-3 drops of 1%

FeCl3. Phenolic was tested by adding 1 mL of longan seed extract was inserted into a reaction tube. Two drops of FeCl3 solution with 5% concentration were added. As a result, a solid green or blue color indicates phenolic content. Lastly steroid/triterpenoid was tested by adding 1 mL of longan seed extract with 2 mL chloroform and shaken vertically and then two drops of concentrated acetic anhydride and sulfuric acid (H₂SO₄) were added to the filtrate.

The making of *P.gingivalis* suspension

Mueller Hinton Broth (MHB) medium at 10.5 g was dissolved in 500 mL of ddH₂O and then medium was heated with a microwave until boiling and homogenous. After the heating process, sterilized the medium with an autoclave at 121°C for 20 minutes. *P.gingivalis* colony cultured in a Mueller Hinton Agar (MHA) medium was inoculated into the MHB medium. The suspension in the reaction tube was made homogenous using a vortex mixer. The turbidity of bacterial fluid isolates was made homogenous for comparison with a standard suspension of McFarland 0.5 to obtain an inoculum with 1.5x10⁸ CFU/mL of bacteria.

Bacterial culture procedure

Nineteen grams of MHA were weighed, inserted into an erlenmeyer flask, and dissolved into 500 mL of ddH₂O. The solution was then stirred and heated in a microwave until boiling to make a homogenous solution. Afterwards, the media were sterilized in an autoclave at 121°C for 20 minutes, then poured into a petri dish. One of pure culture bacteria was planted on the MHA surface with a streak plate method.

Work procedure of well diffusion test

A sterile cotton swab was dipped into a MHB filled with *P.gingivalis* suspension with turbidity adjusted to McFarland 0.5. The cotton swab was spread evenly on the surface of the MHA and kept at 24°C for 3-5 minutes. Wells were made and inserted with 50 mL each of 0.2% chlorhexidine for the positive control, 10% DMSO for the negative control, and longan seed extract with 3.125%, 6.25%, 12.5%, 25%, 50%, 75%, and 100% concentrations in each well. The media were allowed to stand for 3-5 minutes to enable diffusion, then incubated at 37°C for 24 hours.

Clear zones were seen after 24 hours of incubation. The agar well repetition was carried out three times. The inhibition zone was measured with a caliper with the results in millimeters of diameter. Antibacterial activities were measured with a categoric scale using ordinal data with the following categories. An inhibition zone of less or equal to 5 mm is considered weak, an inhibition zone with a diameter between 10-20 mm is considered strong, and an inhibition zone with a diameter

ter of more or equal to 20 mm is considered very strong. This procedure was repeated three times according to the Federer formulation.

RESULTS

Phytochemical screening results

A qualitative phytochemical test on longan seed extract indicated that longan seeds contain bioactive substances such as phenolic, tannin, flavonoid, and triterpenoid.

Table 1 Qualitative phytochemical screening of longan seed extract

Secondary Metabolite	Screening Result
Phenolic	+++
Tannin	+++
Flavonoid	+++
Saponin	-
Triterpenoid	+++
Alkaloid	-

Measurement of inhibition zone diameter of longan seed extract on *P. gingivalis*

Based on the above bar chart, longan seed extracts from 25% concentration showed antibacterial activities on *P. gingivalis* with a mean diameter of 3.15 mm. The inhibition level of longan seed extract is lower than 0.2% chlorhexidine, which was the positive control. The inhibition level of longan seed extract is directly proportional to its concentration. The higher the concentration, the higher the antibacterial activity produced, with the highest concentration of 100% providing 10.07 mm of antibacterial activity.

Statistical Analysis

Non-parametric post hoc test concludes a significant difference, measured from the *sig. column*, which show-

ed that the significance level was lower than the significant value (p-value < 0.05). The 75% and 100% longan seed extract and 0.2% chlorhexidine as positive control showed a significant difference in inhibiting *P. gingivalis*.

DISCUSSION

The inhibition level is marked by clear zones surrounding the well. The higher the clear zone, the higher the inhibition level. Based on the study's results, 100% longan seed extract has a higher inhibition level than other concentrations because it has more antibacterial substances. However, 100% longan seed extract showed a lower inhibition level than 0.2% chlorhexidine, the positive control. The qualitative phytochemical test on longan seed extract showed bioactive substances of phenolic, tannin, flavonoid, and triterpenoid. Phenolics are a very diverse class of polyphenols and are produced by shikimic acid through the phenylpropanoid pathway.¹⁵

Phenolics work by denaturing proteins, destroying bacterial membranes, inhibiting virulence factors and toxins, and suppressing the formation of bacterial biofilms.^{16,17} Phenolics in plants act as signaling molecules during the initiation of plant developmental stages. The mechanism of phenolic acid in inhibiting bacteria is not fully understood due to its complex chemical structure, but the hypothesis states that phenolic acid can damage the electrochemical gradient of the mitochondrial membrane.¹⁸

The mechanism of action of tannins is explained by inhibition of extracellular microbial enzymes, loss of substrates in the growth of a bacterium, or direct action on bacterial metabolism.¹⁹ In gram-negative bacteria,

Table 2 Three repetition measurement of inhibition zone diameter of longan seed extract on *P. gingivalis*

Treatment	Inhibition Zone Diameter			Mean	Std Dev	RSD
	1st repetition	2nd repetition	3rd repetition			
Positive Control (Chlorhexidine 0.2%)	11.98	11.95	11.89	11.94	0.05	0.38
Negative Control (DMSO 10%)	0.00	0.00	0.00	0.00	0.00	0.00
Longan Seed Extract 100%	10.20	9.69	10.33	10.07	0.34	3.36
Longan Seed Extract 75%	7.87	8.12	8.15	8.05	0.15	1.91
Longan Seed Extract 50%	5.67	5.30	5.23	5.40	0.24	4.38
Longan Seed Extract 25%	3.76	3.52	3.24	3.51	0.26	7.42
Longan Seed Extract 12.5%	0.00	0.00	0.00	0.00	0.00	0.00
Longan Seed Extract 6.25%	0.00	0.00	0.00	0.00	0.00	0.00
Longan Seed Extract 3.125%	0.00	0.00	0.00	0.00	0.00	0.00

Table 3 Non-parametric post hoc test

	PC	NC	100%	75%	50%	25%	12,5%	6,25%	3,125%
PC	11.94000*	1.86667	3.89333*	11.94000*	11.94000*	11.94000*	11.94000*	11.94000*	11.94000*
NC	-11.94000*		-10.07333*	-8.04667*	0.00000	0.00000	0.00000	0.00000	0.00000
100%	-1.86667	10.07333*		2.02667*	10.07333*	10.07333*	10.07333*	10.07333*	10.07333*
75%	-3.89333*	8.04667*	-2.02667*		8.04667*	8.04667*	8.04667*	8.04667*	8.04667*
50%	-11.94000*	0.00000	-10.07333*	-8.04667*		0.00000	0.00000	0.00000	0.00000
25%	-11.94000*	0.00000	-10.07333*	-8.04667*	0.00000		0.00000	0.00000	0.00000
12,5%	-11.94000*	0.00000	-10.07333*	-8.04667*	0.00000	0.00000		0.00000	0.00000
6,25%	-11.94000*	0.00000	-10.07333*	-8.04667*	0.00000	0.00000	0.00000		0.00000
3,125%	-11.94000*	0.00000	-10.07333*	-8.04667*	0.00000	0.00000	0.00000	0.00000	

PC = Positive Control (Chlorhexidine 0.2%); NC = Negative Control (DMSO 10%)

tannin activity takes place more slowly than in gram-positive bacteria, this is due to the presence of a lipid bilayer.²⁰ The antibacterial effect of tannins may be mediated by the ability of complex metal ions in the bacterial growth environment.²¹ The results of the study show that tannins can inhibit 50% of biofilm formation.²² Based on research Matin, et al, against *E.coli* bacteria which are gram-negative bacteria showed that there were significant results in the measured inhibition zone, which was 12.5-30.2 mm at a concentration of 50%.²¹

Flavonoids are polyphenolic compounds found in all vascular and avascular plants, these compounds contain high nutritional value.²³ Flavonoids can inhibit nucleic acid synthesis, cytoplasmic function, metabolism, attachment and biofilm formation, inhibit porin, change membrane permeability, and reduce pathogenicity in bacteria.²⁴ In addition, flavonoids can also protect DNA from damage, strengthen capillaries, have anti-inflammatory effects, and protect against radiation. Flavonoids in plants have important roles including as signaling molecules, detoxifying agents, phytoalexins, and assisting in the stimulation of seed germination. Apart from that, flavonoids are also compounds that give aroma, color and taste to fruit, flowers or seeds in a plant.²⁵ The aroma and taste of fruit imparted by flavonoids aid in plant reproduction in attracting pollinators and animals which are responsible for dispersal of fruits and seeds.²⁶

Plant-derived triterpenoids belong to a class of molecules with multiple activities including antimicrobial activity which are the reference for treatment discovery as proven by many reports and even clinical trials by many researchers.²⁷ Triterpenoids react with transmembrane proteins (porins) in bacterial extracellular cell walls and then form polymer bonds that cause damage to the porins.¹⁶ Triterpenoids also increase the permeability of bacterial cells by inserting themselves into the lipid bilayer, thereby influencing the selective permeability of cells and contributing to their antibacterial effect.²⁸

Based on the study, longan seed extract has anti-

bacterial activities, which were established by forming inhibition zones in MHA media inoculated by *P.gingivalis*. Inhibition zones began to start at 25% concentration. However, based on statistical tests, the concentrations that showed significant differences were 75%, 100%, and 0.2% chlorhexidine, the positive control. Longan seed extract at 100% concentration showed the highest mean of all experimental groups compared to other concentrations. No antibacterial activity or weak antibacterial activities might be caused by a lack of bioactive substances in low concentrations to inhibit bacterial growth.²⁹ Zeniusa et al. stated that the diameter of the inhibition zone was affected by the turbidity of the bacterial suspension. If the bacterial suspension has lower turbidity than the standard of McFarland 0.5, the inhibition zone diameter will be more significant. However, the inhibition zone diameter will be smaller if the bacterial suspension has more turbidity than the standard McFarland 0.5.³⁰

Other than bacterial turbidity, temperature and incubation time also affect inhibition zone diameter. The media must be incubated at 37°C for 24 hours.³¹ Based on the study, the higher the concentration of longan seed extract, the higher the inhibition level produced. A previous study on *S.aureus* showed that longan seed extract added with 1% nano chitosan effectively inhibits *S.aureus* at 80% concentration with an inhibition zone of 9.38 mm, 60% with an inhibition zone of 8.47 mm, 40% with an inhibition zone of 7.10 mm. The study concludes that inhibition zone increase is directly proportional to concentration. Other than that, several other studies have also proven its effectiveness with a MIC of 64 µg/mL for *S.aureus*, where MIC of < 500 µg/mL is categorized as strong inhibition. The lower the MIC, the larger the diameter of the inhibition zone.^{9,12} Based on these studies, longan seed extract has a significant antibacterial effect in gram-negative and gram-positive bacteria.

The study concludes that there is an effect of longan seed extract in inhibiting the growth of *P.gingivalis*.

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