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Extract wuluh starfruit (*Averrhoa bilimbi* L.) potentiate the effect of antibacterial against *Porphyromonas gingivalis*

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Abstract
Background: Periodontitis is a problem in dental and oral health with a high prevalence in Indonesia of 74.1%. *Porphyromonas gingivalis* was found in 85.75% of the subgingival plaque of periodontitis patients. Metronidazole is often used as adjunctive therapy to provide maximum results in the treatment of periodontitis but is contraindicated in certain patients. The wuluh starfruit has been shown to have active compounds including tannins, saponins, alkaloids, and flavonoids as well as vitamins that are antibacterial. This study aimed to analyze the antibacterial power of wuluh starfruit extract on the growth of *P. gingivalis* at concentrations of 6.25%, 12.5%, 25%, 50%, and 100%.
Methods: This type of research is an experimental laboratory with the post-test only control group design. Antibacterial test using disc diffusion method. This study consisted of 5 treatment groups (concentration of wuluh starfruit extract 6.25%, 12.5%, 25%, 50%, and 100%) and 2 control groups (negative control group and positive control group).
Results: The research data in the form of the diameter of the inhibition zone were analyzed by the Kruskal Wallis test followed by the Mann-Whitney test with the results that there were significant differences.
Conclusions: Based on the research, it can be concluded that the extract of wuluh starfruit (*Averrhoa bilimbi L.*) at concentrations of 6.25%, 12.5%, 25%, 50%, and 100% had antibacterial activity against the growth of *P. gingivalis*, the smallest at a concentration of 6.25% and the largest at a concentration of 100%.

Keywords: Antibacterial, wuluh starfruit, *Porphyromonas gingivalis*

Introduction
Dental and oral health is a state of health of the hard tissues and soft tissues of the oral cavity that enables individuals to eat, speak and interact socially without dysfunction, aesthetic disturbances and discomfort due to disease, occlusion deviation and tooth loss so that they are able to live socially and productively [1]. The Global Burden of Disease Study (2019) estimates that dental and oral diseases affect around 3.5 billion people worldwide. The same study states that there are 1.1 billion cases of periodontitis globally and there has been an increase of 8.44% from 1990 to 2019 worldwide, especially in developing countries [2]. The prevalence of periodontitis in Indonesia shows a percentage of 74.1% [1].
Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by certain microorganisms which results in progressive destruction of the periodontal ligament and alveolar bone with increased formation of probing depth, gingival recession, or both [3]. The main etiology of periodontitis is plaque bacteria. Research shows that 85.75% of *Porphyromonas gingivalis* bacteria are found in subgingival plaque from patients with periodontitis [4]. The process of *P. gingivalis* invasion uses a panel of virulence factors that cause dysregulation of innate immune and inflammatory responses [5]. The virulence factors of *P. gingivalis* include the capsule, fimbriae, gingipain and lipopolysaccharide (LPS) which play the most important role in inducing periodontitis [6].
Someone with periodontitis if not treated properly can cause tooth loss (7). Bacterial infections can be overcome by giving antibiotics as a supporting therapy to provide maximum results in the treatment of periodontitis.

Metronidazole gel is often used as an effective local antibiotic in cases of periodontitis. However, this antibiotic is contraindicated in pregnant, lactating patients and hypersensitivity to metronidazole. This becomes the basis for utilizing natural ingredients as an alternative, one of which is using wuluh starfruit. The wuluh starfruit plant has many benefits in daily uses, including in the health sector. The parts of the wuluh starfruit plant that can be utilized are the flowers, fruits, leaves and stems [8]. Wuluh starfruit is often used by the community as a food ingredient and traditional medicine, such as for coughs, canker sores, acne, hypertension, diabetes, mumps, rheumatism, muscle aches, and tinea versicolor [9].

Phytochemical tests showed that wuluh starfruit contains active chemical compounds such as flavonoids, alkaloids, tannins and saponins which are antibacterial [10]. Wuluh starfruit is known to also contain vitamins such as vitamin A, beta-carotene, thiamin, riboflavin, and niacin which have been studied and believed to be not only antioxidants but also have antibacterial abilities [11].

The potential of wuluh starfruit to inhibit bacterial growth has been studied before. Research by Rahmiati *et al*. (2017) who tested the inhibition ability of starfruit extract on the growth of *Streptococcus mutans* with varying concentrations of 25%, 50%, 75% and 100%, found that at a concentration of 25% it was still able to inhibit the growth of *S. mutans*. Another study with the same extract but with different concentration variations, namely 6.25%, 12.5%, 25%, and 50% showed that at the lowest concentration of 6.25% it was still able to inhibit the growth of the bacteria *methicilin Resistant Staphylococcus aureus* [12]. Research on the ability of wuluh starfruit extract to inhibit the growth of *P. gingivalis* bacteria has never been done before. This background motivated the authors to research the potential antibacterial of wuluh starfruit extract on the growth of *P. gingivalis*, especially at concentrations of 6.25%, 12.5%, 25%, 50% and 100%.

Materials and Methods

This research was approved by the Health Research Ethics Commission (KPEK) Faculty of Dentistry, Jember University No. 1441/UN25.8/KPEK/DL/2022. This type of research was laboratory experimental with the post-test only control group design to find out the difference between the treatment group and the control group. The treatment group consisted of wuluh starfruit extract concentrations of 6.25%, 12.5%, 25%, 50% and 100%. The control group consisted of a positive control (metronidazole gel) and a negative control (sterile distilled water).

The tools used in this study included: uninsulated petri dishes, oven, digital caliper, micropipette, rotary evaporator, maceration vessel, autoclave, vortex, blender, desiccator, incubator, bio safety cabinet, rack and test tube. The materials used included: pure culture of *P. gingivalis* bacteria, fresh wuluh starfruit, blood agar plate media, 96% ethanol, 25% metronidazole gel, sterile paper discs, sterile distilled water, filter paper, and cotton swabs.

The wuluh starfruit fruit used has been identified at the UPT. Integrated Agricultural Development, Jember State Polytechnic. Wuluh starfruit is extracted by maceration method. Wuluh starfruit that has been washed and thinly sliced is dried in an oven with a temperature of 45ºC for 4 days and then mashed using a blender to obtain simplicia. The simplicia was macerated using 96% ethanol with a ratio of 1:5 simplicia and solvent. The filtrate was evaporated using a rotary evaporator at 70 ºC. After that, dilution of wuluh starfruit extract was carried out using the serial dilution method using sterile distilled water as a solvent to concentrations of 6.25%, 12.5%, 25%, 50% and 100%.

The antibacterial potential test was carried out using the disc diffusion method. Each paper disc is dripped with 20 µl of the test substance. Paper discs were then placed on the surface of the blood agar plate which had been inoculated with *P. gingivalis* by streaking method. The media was then put into a desiccator and incubated at 37ºC for 24 hours. Measurement of the diameter of the inhibition zone was carried out after 24 hours of incubation using a digital caliper by 3 different observers and then the measurement results were averaged.

Results

The results of this study can be seen in Table 1 and Figure 1 with the average diameter of the inhibition zone and the standard deviation of each study group.

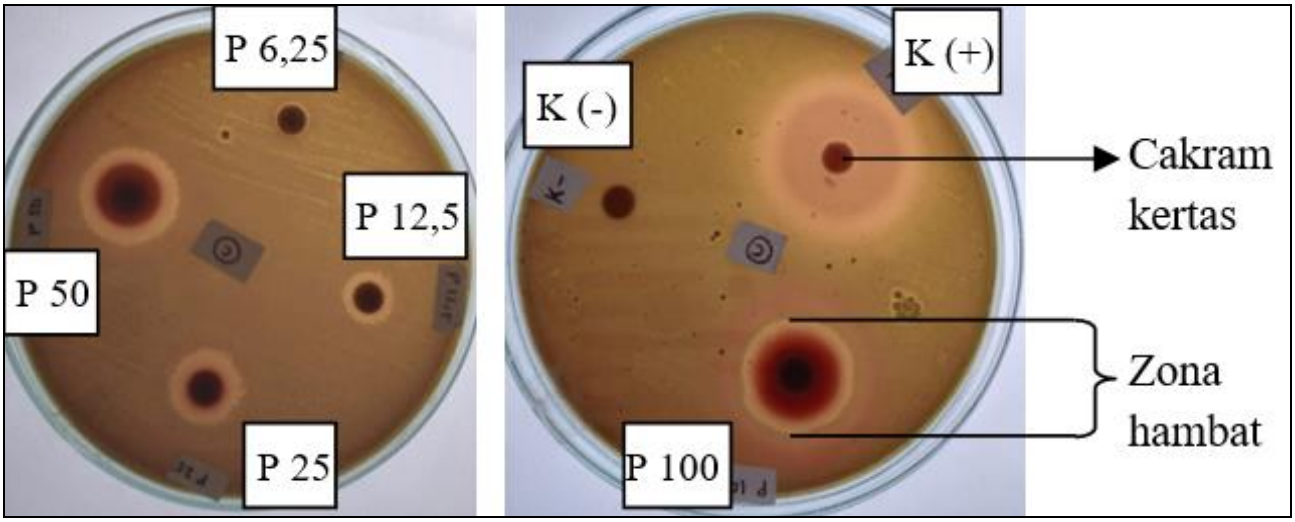

Fig 1: Samples of experiment results

Table 1: Average and standard deviation of the diameter of the inhibition zone (mm) on the growth of *P. gingivalis* bacteria

Group	N	Average diameter of the inhibition zone	Standard Deviation
Wuluh Extract 6,25%	4	6.92 mm	± 0.29
Wuluh Extract 12,5%	4	8.41 mm	± 1.34
Wuluh Extract 25%	4	11.67 mm	± 1.92
Wuluh Extract 50%	4	17.01 mm	± 3.33
Wuluh Extract 100%	4	21.18 mm	± 1.59
Positive control	4	37.83 mm	± 4.14
Negative control	4	0.00 mm	0

Data analysis began with the Shapiro Wilk normality test, which obtained a significance value for all study groups greater than 0.05 (p>0.05), indicating that the data were normally distributed. Homogeneity test with the Levene Test obtained a significance value of less than 0.05 (p<0.05) so that the data was considered not homogeneous. Based on these results, the data were then analyzed using non-parametric tests, namely the Kruskal Wallis test and the Mann-Whitney test.

The Kruskal Wallis test showed a significance value of 0.000 (p<0.05) so it was concluded that there were differences in the diameter of the inhibition zone between the study groups. Further analysis of the data was carried out using the Mann-Whitney test to find out which study group had a significant difference. The results of the Mann-Whitney test showed that in wuluh starfruit extract concentrations of 6.25%, 12.5%, 25%, 50% and 100% there were significant differences between concentrations with the largest inhibition zone diameter at 100% concentration and the smallest at 6.25%. The results of the Mann-Whitney test between all study groups showed a significant difference in the diameter of the largest inhibition zone in the positive control group and the smallest in the wuluh starfruit extract group with a concentration of 6.25%.

Discussion

Based on the results of the research that has been analyzed, it proves that wuluh starfruit extract has antibacterial power against *P. gingivalis*. This was indicated by the presence of inhibition zones around the paper discs in all groups of wuluh starfruit extract concentrations of 6.25%, 12.5%, 25%, 50% and 100%. The inhibition zone formed is a form of the role of the nutrient content and active compounds in the wuluh starfruit which are able to inhibit the growth of *P. gingivalis* bacteria. Phytochemical tests on wuluh starfruit extract gave positive results for the presence of active compounds such as saponins, flavonoids, tannins, and alkaloids. These compounds are known to have the ability to inhibit bacterial growth [13, 14].

Saponins are antibacterial agents that are bactericidal because they disrupt the cytoplasmic membrane. The mechanism of action of saponins in inhibiting bacterial growth is by interfering with the surface tension of the cell wall by diffusing through the outer membrane and cell wall and then binding to the cytoplasmic membrane. This activity causes the cytoplasm to leave the cell and cell lysis occurs [15, 16].

The active compounds of flavonoids form complexes with bacterial proteins through hydrogen bonds which result in the structure of the cell wall and the bacterial cytoplasmic membrane becoming unstable. This will disrupt the permeability function of the bacterial cell resulting in bacterial cell death (Saputra and Anggraini, 2016) [17]. Flavonoids also limit the use of bacterial oxygen to inhibit the energy metabolism needed by bacteria for macromolecular biosynthesis [15].

Tannins are able to inhibit the growth of various microorganisms because they are growth inhibitors. The mechanism of action of tannins is facilitated by the lysis of the bacterial cell wall due to the action of flavonoids and saponins. The antimicrobial action of tannins takes place through several mechanisms, namely reacting with bacterial cell membranes to inactivate microbial adhesins and inactivation of hydrolytic enzymes such as proteases and carbohydrolases and inhibiting enzymes in envelope transport proteins (Pratiwi *et al*., 2015; Saputra and Anggraini, 2016) [18, 17].

Other active antibacterial compounds in wuluh starfruit that play a role in inhibiting the growth of *P. gingivalis* are alkaloids. Alkaloids lyse bacteria by destroying the bacterial cell wall layer due to the peptidoglycan component of the bacterial cell being disrupted by the alkaloids. Another mechanism of alkaloids in inhibiting bacterial growth is known that alkaloids act as DNA interchelators and inhibit bacterial topoisomerase enzymes (Kurniawan and Aryana, 2015; Rijayanti, 2014) [19, 15].

Wuluh starfruit also contains vitamin A, beta-carotene, thiamin, riboflavin, niacin, and vitamin C which have been studied and have antibacterial properties [11, 20–22]. The mechanism of direct antibacterial action of the above vitamins is still not known with certainty but there is a relationship between the antioxidant and antibacterial properties of natural chemical compounds. Antibacterial and antioxidant activity in inhibiting bacterial growth includes three basic mechanisms including disrupting the permeability of the outer membrane, cytoplasmic leakage, and inhibiting the formation of nucleic acids (Karpiński and Adamczak, 2019) [23].

The diameter of the inhibition zone showed a significant difference between all study groups. The negative control did not form an inhibition zone around the paper discs, so it was concluded that sterile distilled water had no antibacterial effect on the growth of *P. gingivalis*. The results of the difference test between the negative control and 6.25% concentration of wuluh starfruit extract showed a significant difference. This indicates that 6.25% concentration of wuluh starfruit extract has antibacterial activity against *P. gingivalis*.

The results of the Mann-Whitney test showed a significant difference between the concentrations of wuluh starfruit extract. Wuluh starfruit extract at a concentration of 100% showed the largest inhibition zone diameter of 21.18 mm and the smallest inhibition zone diameter at a concentration of 6.25%, namely 6.92 mm. This is in accordance with the statement of Brooks *et al*., (2007) which states that the effectiveness of antimicrobial substances is influenced by the concentration of substances used. The amount of concentration of an ingredient will affect the amount of active ingredient content [24].

The largest diameter of the inhibition zone was shown by the positive control, namely metronidazole gel. The ability to inhibit the growth of *P. gingivalis* bacteria was greater than the 100% concentration of wuluh starfruit extract, possibly

because metronidazole is a broad spectrum antibiotic and is known to be effective against anaerobic bacteria. The selectivity of metronidazole against anaerobic micro-organisms is due to the redox potential of its electron transport component which is responsible for redoxing nitro groups and producing toxic metabolites against bacteria [25]. The low molecular weight of the compound makes it easier for metronidazole to diffuse across the bacterial cell membrane as a prodrug that is activated in the bacterial cytoplasm. This antibiotic becomes cytotoxic and interacts with DNA causing the loss of the DNA helix structure thereby inhibiting bacterial DNA synthesis [26].

Conclusion

Based on the results of the research that has been carried out, it can be concluded that wuluh starfruit extract (*Averrhoa bilimbi L.*) concentrations of 6.25%, 12.5%, 25%, 50%, and 100% have antibacterial power against the growth of *P. gingivalis*, the smallest at a concentration of 6.25% and the largest at a concentration of 100%.

Conflict of Interest

Not available

Financial Support

Not available

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