

## Effect of Jamblang (*Syzygium Cumini*) Fruit Extract on Alveolar Bone Resorption in Wistar Rats Induced with Chronic Periodontitis

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### Abstract

Chronic periodontitis is an infectious disease that results in inflammation by bacteria that causes progressive damage to the supporting tissues of the teeth, especially the alveolar bone. Jamblang fruit (*Syzygium cumini*) is an herbal plant that contains flavonoids, phenols, terpenoids, tannins, and vitamin C compounds that have potential as antibacterial, anti-inflammatory, and antioxidant.

This study aims to determine the effect of jamblang fruit extract on alveolar bone resorption in Wistar rats induced by chronic periodontitis.

Induction of chronic periodontitis was performed for 14 days by placing a 0.008 inch wire in the cervical area and tied to the submarginal part of the mandibular central incisor. The study was conducted using posttest with control group design and divided into four groups: negative control group (treated with distilled water), positive control group (treated with metronidazole), treatment group 1 (treated with 50% jamblang fruit extract), and treatment group 2 (treated with 75% jamblang fruit extract). Treatments were topically applied in the morning and evening with a difference of seven hours for 14 days.

Kruskal-Wallis analysis showed a significant difference in the reduction of alveolar bone resorption clinically and radiographically with  $p=0.020$  and  $p=0.041$  ( $p<0.05$ ). Conclusion: It is concluded that jamblang fruit extract (*Syzygium cumini*) has an effect on reducing alveolar bone resorption in *Rattus norvegicus* induced chronic periodontitis.

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### Introduction

Periodontitis is caused by a group of microorganisms that cause inflammation of the periodontal tissues and has a wide spread rate.<sup>1</sup> Research on the epidemiology of periodontitis has been conducted. The percentage of periodontitis cases in Indonesia based on Basic Health Research (RISKESDAS) data in 2018 reached 74.1%.<sup>2</sup> Periodontitis is divided into chronic periodontitis and aggressive periodontitis. Chronic periodontitis is periodontitis that often occurs in adulthood, but can be occurred in childhood.<sup>3</sup>

Chronic periodontitis is the result of a correlation between plaque and various

bacteria.<sup>4</sup> Gram-negative anaerobic bacteria are a group of bacteria that are commonly found in chronic periodontitis. *Porphyromonas gingivalis* is one of the Gram-negative black-pigmented anaerobic bacteria that invade the subgingiva and has been identified as one of the main pathogens of periodontal disease. In a study by Zhang et al observed that *Porphyromonas gingivalis* invaded alveolar bone in a periodontitis-induced mice model.<sup>5</sup> The bacteria will release Lipopolysaccharide toxin (LPS) which causes activation of inflammatory cells and results in the release of cytokines and local factors.<sup>6,7</sup> These factors will stimulate activity in osteoclasts and preosteoclast, resulting in an increase in osteoclast cell groups that function to resorb bone.<sup>8</sup>

Periodontitis treatment can be addressed by removing local factors by scaling and root planing, instructions to maintain oral hygiene, and administration of antibacterial drugs.<sup>9</sup> Treatment of chronic periodontitis with

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antibacterial therapy, especially metronidazole, is indicated because it has been shown to be effective against Gram-negative anaerobic bacteria in chronic periodontitis.<sup>10</sup> However, the use of antibacterial drugs has side effects, especially on the digestive tract, skin redness, headache, nausea, and dry mouth.<sup>10</sup> Many studies have been conducted to get other alternative treatments by utilizing herbal plants that have minimal side effects and can help cure chronic periodontitis, one of which is Jamblang fruit.<sup>11</sup>

According to previous research, jamblang fruit contains high antioxidants from anthocyanin compounds, flavonoids, and polyphenol groups such as tannins.<sup>12</sup> These compounds have potential as antibacterials, able to inhibit cell wall formation, cause damage to cells, and inhibit energy metabolism of bacteria.<sup>13</sup> According to Akhila's research jamblang fruit also contains several minerals such as calcium, potassium (Ca, K), and vitamins (B-complex, vitamin C).<sup>14</sup>

Minerals and vitamins are contained in jamblang fruit have important roles and functions for the body, both in cells, tissues, and organs which can function in the maintenance of bones and teeth.<sup>15</sup> This is supported with previous study that vitamin C in addition to being useful as an antioxidant is also an important requirement for collagen formation needed for growth and repair throughout the body such as wound healing and repair of bones and teeth.<sup>16</sup>

Based on the above description, jamblang fruit extract may have potential in alveolar bone repair in chronic periodontitis. Thus, researchers are interested to know the effect of jamblang fruit extract on alveolar bone resorption in Wistar rats induced by chronic periodontitis.

## Materials and methods

The research was conducted in November 2020 at the Pharmacology Laboratory of the Faculty of Veterinary Sciences, Syiah Kuala University to conduct extraction and phytochemical testing of jamblang fruit (*Syzygium cumini*). Induction of chronic periodontitis, administration of extracts and clinical observations of *Rattus norvegicus* were carried out at the Laboratory of Animal Experiments of the Faculty of Veterinary Sciences, Syiah Kuala University and observation of alveolar bone of *Rattus*

*norvegicus* through X-rays was carried out at the Laboratory of the Faculty of Dentistry, Syiah Kuala University, and at the Laboratory of the Faculty of Mathematics and Natural Sciences, Syiah Kuala University to conduct vitamin C tests of jamblang fruit extract

The method of work in this study was first the manufacture of *Syzygium cumini* extract is done by maceration technique using ethanol solvent. Furthermore, phytochemical tests and vitamin C tests were carried out. Then the animals were adapted for one week in the laboratory before being given treatment and divided into four groups, which were the Negative Control group, Positive Control group, Treatment Group 1, and Treatment Group 2. Next, a 0.008 inch wire was placed in the cervical area and tied to the submarginal part of the central incisor of the lower jaw. The wire was left in place for 11 days

Then Metronidazole was applied to tested animals for the Positive Control group, distilled water for the Negative Control group, 50% concentration of jamblang fruit extract for Treatment Group 1, and 75% concentration of jamblang fruit extract for Treatment Group 2.

## Data Analysis

Data analysis was carried out using SPSS and hypothesis testing was carried out using the one way Anova test and then the Least Significant Different (LSD) further test was carried out to determine significant differences.

## Results

The results of the phytochemical test showed that the *Syzygium cumini* extract in this study contained flavonoids, phenols, terpenoids, and tannins (Table 1). Furthermore, the results of the vitamin C test through Iodometri (Titration) found that *Syzygium cumini* extract in this study has a vitamin C content of 8.01 mg/100 g.

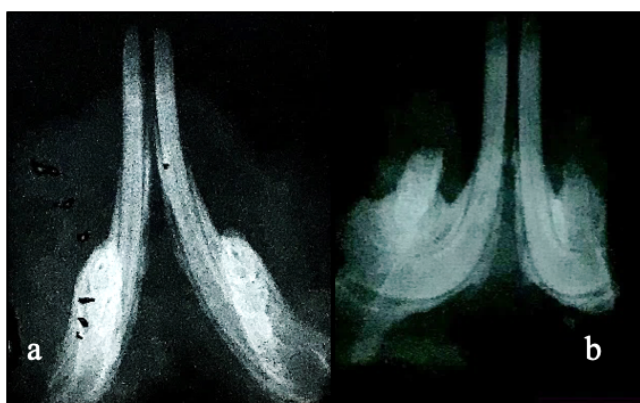
Properties of Compounds	Reagent	Result
Flavonoid	Mg and HCL	+
Fenol	FeCl <sub>3</sub>	+
Terpenoid	Liebermann-Burchard	+
Steroid	Liebermann-Burchard	-
Saponin	Aquades	-
Tannin	FeCl <sub>3</sub>	+
Alcaloid	Dragendorff	-
	Mayer	-
	Wagner	-

**Table 1.** Phytochemical test of *Syzygium cumini* extract.

The results of periodontitis manipulation for 14 days using wire, clinically visible signs of redness, bleeding during probing and recession of the gingiva (Figure 1). Radiographic examination showed alveolar bone resorption as a sign of chronic periodontitis (Figure 2).



**Figure 1.** Manipulation process of chronic periodontitis. a) Wire placement and b) Plaque build-up and bleeding during probing.



**Figure 2.** Radiographic image of the mandible of Wistar mice. (a) Normal image of the mandible of male Wistar mice, the arrow shows the peak of the alveolar bone (b) Image of periodontitis. The arrow shows the decrease of alveolar bone in the interdental part of the mice teeth.

Following treatment for 14 days, a clinical examination was conducted by measuring the depth of periodontal pockets using a prob. The average depth of chronic periodontitis pockets of each *Rattus norvegicus* in each group was summed and divided by three (Table 2).

The table above shows the average value of *Rattus norvegicus* poket depth after 14 days of treatment after application of metronidazole, distilled water, 50% and 75% jamblang fruit extract. The table shows that in the positive group (Metronidazole) the socket depth became

0.83 mm, in the negative group (distilled water) the socket depth became 4.33 mm, then in treatment group 1 (50% jamblang fruit extract) the socket depth became 2.66 mm, and in treatment group 2 (75% jamblang fruit extract) the socket depth became 0.93 mm.

Result shows that there is a significant difference ( $p < 0,05$ ) in the decrease in *Rattus norvegicus* socket depth in all groups. Furthermore, Table 3 shows the results of the *Post Hoc Dunn's Test* showed the results of comparisons between treatment groups 1 and positive control, treatment 2 and positive control, treatment 2 and treatment 1, treatment 1 and negative control did not show a significant decrease in socket depth. While comparisons between treatment group 2 and negative control, positive control group and negative control showed a significant decrease in socket depth.

Group	Before Treatment (mm)	After Treatment (mm)
Control Positive (Metronidazole)	3.50	0.83
Control Negative(Aquades)	4.50	4.33
Treatment 1 (Syzygium cumini extract 50%)	3.83	2.66
Treatment 2 (Syzygium cumini extract 75%)	3.33	1.33

**Table 2.** Average Depth of Chronic Periodontitis Pockets of *Rattus norvegicus*.

Group	Sig.	Description
Treatment 1 – Control Positive	0.072	Not significance
Treatment 2 – Control negative	0.524	Not significance
Treatment 2 – Treatment 1	0.246	Not significance
Treatment 1 – Control negative	0.271	Not significance
Treatment 2 – Control negative	0.024	Significance
Control Positive – Control negative	0.004	Significance

**Table 3.** Result of Post Hoc Dunn's Test.

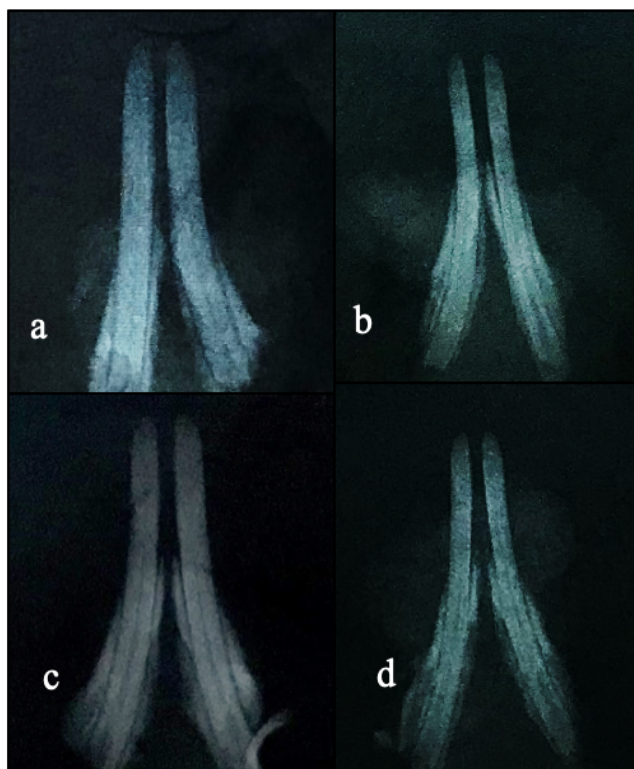
After 14 days of treatment, radiographic examination was also carried out to measure alveolar bone resorption. The results of measuring alveolar bone resorption from each group with an average can be seen in Table 4.

Group	Average Measurement (mm)
Control negative (Aquades)	4
Control Positive (Metronidazole)	0.83
Treatment I (Syzygium cumini extract 50%)	2
Treatment II (Syzygium cumini extract 75%)	1.66

**Table 4.** Measurement of Alveolar Bone Resorption in Control and Treatment Groups.



Table 4 shows that the highest average alveolar bone resorption measurement results are in the negative control group of 4 mm, then in the positive control group of 0.83 mm, treatment group 1 of 0.56 mm, and in treatment 2 has alveolar results of 1.66 mm.



**Figure 3.** Radiographic Images of Chronic Periodontitis in *Rattus norvegicus*, (a) Negative Control, (b) Positive Control, (c) 50% Extract Treatment, (d) 75% Extract Treatment.

The results of bone resorption measurements showed that there was a significant difference in the decrease in bone resorption in *Rattus norvegicus* induced chronic periodontitis in all groups with a value of  $p = 0.041$  ( $p < 0.05$ ). *Post Hoc Dunn's Test* (Table 5) shows a comparison between treatment group 1 and positive control, treatment 2 and positive control, treatment 2 and treatment 1, treatment 1 and negative control. On the other hand, treatment group 2 and negative control did not show a significant reduction in alveolar bone resorption. On the other hand, the comparison between the positive control and negative control groups showed a significant reduction in alveolar bone resorption.

Group	Sig.	Description
Treatment 1 – Control Positive	.163	Not significance
Treatment 2 – Control negative	.269	Not significance
Treatment 2 – Treatment 1	.771	Not significance
Treatment 1 – Control negative	.146	Not significance
Treatment 2 – Control negative	.081	Not significance
Control Positive – Control negative	.004	Significance

**Table 5.** Comparison between treatment groups.

## Discussion

This study aims to determine the effect of jamblang fruit extract (*Syzygium cumini*) on alveolar bone resorption in Wistar mice induced by chronic periodontitis. The extraction process of jamblang fruit uses maceration method because the advantage of this extraction method is easy and does not need heating so it is less likely that the extract will be damaged or decomposed.<sup>17</sup> Based on compound analysis using phytochemical test, it is found that jamblang fruit extract (*Syzygium cumini*) contains several active compounds such as flavonoids, phenols, terpenoids, and tannins.

Assignment of vitamin C content was done by using an iodometric method (titration) because it is cheap, simple, and does not require sophisticated laboratory equipment. This technique uses iodine as an oxidizer that oxidizes vitamin C and uses amylum as an indicator.<sup>18</sup> The results of vitamin C analysis in mg/100 g showed a result of 8.01 which shows that jamblang fruit extract contains high vitamin C. This is in accordance with research found that jamblang fruit is rich in vitamin C.<sup>12</sup> In contrast, the active compounds contained in jamblang fruit are flavonoids, steroids, saponins, tannins, and alkaloids. The difference in content in jamblang fruit extracts can be caused by differences in plant origin, particle size, and material properties in plants, processing levels, and humidity.<sup>19</sup>

This study used male *Rattus norvegicus* mice because they have a physiological and metabolic system similar to humans and are male so that they are not influenced by hormonal factors.<sup>20</sup> Several studies on alveolar bone resorption have been conducted using mice because they have genomes that are almost similar or homologous to humans and the periodontal anatomy of mice is similar to human periodontal anatomy so that mice are used as animal models for periodontal disease.<sup>21</sup>

The induction of chronic periodontitis in *Rattus norvegicus* was carried out for 14 days by wrapping a *wire ligature* in the cervical area and tied to the submarginal part of the central incisor of the lower jaw.<sup>22</sup> The function of the *wire ligature* installation is to help the plaque buildup process so that it helps accelerate the inflammatory process caused by bacteria in periodontal tissue.<sup>23</sup>

On the 11th day of chronic periodontitis induction, bone loss was not very visible after radiographic examination, but clinically there was inflammation of the gingiva, plaque build-up, and bleeding during probing. This is different from research conducted by Andayani et al that induction of chronic periodontitis in Wistar mice can occur on day 11.<sup>24</sup> The possibility of no bone loss on day 11 is because the ligation technique in mice is not well explored, the operator's experience is lacking so that the fixation of *wire* ties is not correct and easily detached.<sup>25</sup>

Based on the results, all *Rattus norvegicus* in each group developed chronic periodontitis on day 14. Clinical and radiographic observations were made on day 14 after treatment. Research conducted by Ionel et al after 14 days of induction of periodontitis using wire ligature showed clinical tooth mobility, gingival bleeding during probing and bone damage seen through radiographic examination.<sup>19</sup>

In this study, the decrease in alveolar bone resorption can be seen clinically through the examination of pocket depth using periodontal probes before and after treatment. The average depth of periodontal pockets after 14 days of treatment in the negative control group was 4.33 mm, the positive control group was 0.83 mm, treatment group 1 (50% jamblang fruit extract) was 2.66 mm, and treatment group 2 (75% jamblang fruit extract) was 1.33 mm which showed a shallowing in the depth of periodontal pockets.

Based on the results of statistical analysis showed that the positive group, negative group, 50% and 75% concentration of jamblang fruit extract group clinically significant effect on reducing alveolar bone resorption ( $p < 0.05$ ). The potential of jamblang fruit extract (*Syzygium cumini*) in reducing the depth of pockets indicates a decrease in alveolar bone resorption because it contains active compounds such as flavonoids, phenols, terpenoids, and tannins. Flavonoids and

tannins are phenol group compounds that work as antibacterials by inhibiting bacterial nucleic acid synthesis. The inhibition occurs due to disruption of hydrogen binding to nucleic acid bases so that DNA and RNA processes are inhibited.<sup>22</sup>

Flavonoids can also inhibit bacterial growth by destabilizing the cell membrane and energy metabolism of bacteria where there is a change in the hydrophilic and hydrophobic properties of the cell membrane resulting in reduced cell membrane fluidity and resulting in impaired fluid exchange in the cell so that it will have an impact on bacterial cell death.<sup>27</sup> Flavonoids have antimicrobial activity on some bacteria, such as anaerobic gram-negative bacteria that act to inhibit lipid peroxidation, without the activity of anaerobic gram-negative bacteria, the inflammatory process can be inhibited.<sup>28</sup> Besides having antibacterial effects, flavonoids also have anti-inflammatory activity.<sup>23</sup>

In addition to flavonoids, other compounds that have antibacterial effects are terpenoids which can also inhibit bacterial growth.<sup>27</sup> Another compound contained in jamblang fruit extract as antibacterial is tannin. Tannins in low concentrations can inhibit bacterial growth, while in high concentrations can act as antibacterials by coagulating bacterial protoplasm so that a stable bond is formed with bacterial proteins.<sup>23</sup>

Statistical tests showed no significant difference between the depth of periodontal pockets in treatment group 1 and treatment group 2 after treatment. This shows that there is no significant difference between 50% and 75% concentration of *Syzygium cumini* extract.

Radiographic examination conducted after treatment showed a decrease in alveolar bone resorption when compared to the results of bone measurements before treatment. Based on the results of statistical analysis showed that the positive group, negative group, treatment group 1 (50% jamblang fruit extract), and treatment group 2 (75% jamblang fruit extract) radiographically there were significant differences in the decrease of alveolar bone resorption with  $p = 0.041$  ( $p < 0.05$ ).

The average measurement of alveolar bone resorption after 14 days of treatment in the negative control group was 4 mm, the positive control group was 0.83 mm, treatment group 1 was 2 mm, and treatment group 2 was 1.66 mm. The lowest average measurement of alveolar

bone resorption is in the positive control group (*metronidazole*) which means a decrease in alveolar bone resorption. The decrease in alveolar bone resorption in the positive group can be caused by the administration of *metronidazole* which is a broad-spectrum antibiotic and is active against anaerobic bacteria that cause chronic periodontitis. *Metronidazole* has an oral effect on the sub-gingival microbiota and can penetrate the gingival sulcus fluid.

The negative control group had a higher mean than the 50% and 75% *Syzygium cumini* extract treatment groups. This shows that *Syzygium cumini* extract treatment has a therapeutic effect by decreasing alveolar bone resorption which is characterized by higher alveolar bone height compared to the negative control group. This can occur because 1 mouse day cycle is equal to 27 human days, so that 3 months of mice age is the same as human age 6-12 years where there is a growth period.<sup>30</sup> During growth, hormones work directly on osteoblasts with hormone receptors to stimulate increased synthesis of collagen, *osteocalcin* and alkaline phosphatase which increase IGF-I and II which stimulate osteoblast proliferation and differentiation so that tissue growth can take place properly.<sup>31</sup> Another factor is that the higher the concentration, the more active compound content in *Syzygium cumini* extract and the greater its ability to inhibit bacterial growth.<sup>32</sup>

The high level of vitamin C in *Syzygium cumini* also plays a role in stimulating type I collagen synthesis, alkaline phosphatase activity, osteoblast accumulation and matrix mineralization in osteoblasts. Vitamin C also stimulates calcium absorption, can maintain bone mass by stimulating the formation of osteoblasts by forming new bone and suppressing osteoclasts in resorbing bone.<sup>33</sup> While the average alveolar bone resorption of the 50% concentration *Syzygium cumini* extract group showed no significant difference from the 75% concentration *Syzygium cumini* extract treatment group because the two groups showed a decrease in alveolar bone resorption which was not much different. The insignificant difference was due to variations in this study, in the process of preparing jamblang to be made into extracts, it was not done from one time because the jamblang fruit used was too much.<sup>34</sup> The various components in *Syzygium cumini* extract have an effect on reducing alveolar bone resorption due

to the content of antibacterial and anti-inflammatory compounds such as flavonoids, phenols, terpenoids, and tannins that inhibit bacterial growth so that the inflammatory process is also inhibited. Based on this analysis, it can be concluded that jamblang fruit extract (*Syzygium cumini*) with a concentration of 50% and 75% is able to reduce alveolar bone resorption in *Rattus norvegicus* induced chronic periodontitis.

## Conclusions

Based on the study, jamblang fruit extract (*Syzygium cumini*) has an effect on reducing alveolar bone resorption in *Rattus norvegicus* induced chronic periodontitis.

## Declaration of Interest

The authors report no conflict of interest.

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