

# Antibacterial Activity of Sappan Wood (*Caesalpinia sappan L.*) against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*

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## ABSTRACT

The aim of this study was to find the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of sappan wood ethanol extract (*Caesalpinia sappan L.*) toward the growth of *A. actinomycetemcomitans* and *P. gingivalis*. The randomized post test only control group design was applied to this study. The sappan wood ethanol extract was conducted with maceration method using 96% ethanol. Diluted to 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, and 0.39% concentration. The MIC and MBC values of sappan wood ethanol extract toward *A. actinomycetemcomitans* and *P. gingivalis* was then known via the evaluation for Colony Forming Units (CFUs) in MH medium. The MIC and MBC of sappan wood ethanol extract toward *A. actinomycetemcomitans* and *P. gingivalis* was at 1.56% and 3.125% concentration. The ethanol extract of sappan wood reduces the

number of bacterial colonies significantly at  $p=0.00$ . This study concluded that the growth of *A. actinomycetemcomitans* and *P. gingivalis* can be inhibited by sappan wood ethanol extract (*Caesalpinia sappan L.*) at MIC 1.56% and MBC 3.125% concentration.

**Keywords:** Sappan wood ethanol extract, MIC 1.56%, MBC 3.125%, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*.

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## INTRODUCTION

Periodontal disease is a chronic inflammatory disease that affects the gum tissue and bone supporting the teeth, which is indicated by gingival inflammation and periodontal pockets (periodontitis) and the second most common dental disease suffered by the world population. Periodontitis is a chronic infectious disease caused by microorganisms. Its key features include periodontal pocket formation, loss of connective tissue attachment, alveolar bone resorption, and gingival inflammation. The studies states that periodontal disease occurs in 20-50% of the entire population, and can increase the risk of cardiovascular disease by 19%; this risk increases by 44% at the 65 years old.<sup>1,2,3</sup> It is not yet known for sure of the complications that can happen in patients with untreated caries or periodontal disease. Those conditions can cause pain, distinction, bad appearance, and disruption of everyday activity.<sup>4,5</sup> Periodontitis can be treated mechanically, surgically, and with supportive treatment. *Chlorhexidine* is one of the mouthwashes often recommended for supportive treatment to treat periodontal disease because of its antibacterial and antiplaque effect. Extended usage of *chlorhexidine* could lead toward changes in tooth color and dorsal part of the tongue, increasing the buildup of calculus, change the taste perception and drying of the oral mucosa.<sup>6,7</sup> Antibiotic can be used as a supportive treatment because of its ability to decrease the bacterial growth that still exists after mechanical therapy. But, even so, the inaccurate and extended administration of antibiotic could lead to bacterial resistance, this has created the need for a therapy using natural ingredients with antibacterial effects to be developed.<sup>8,9</sup>

Sappan (*Caesalpinia sappan L.*) is a plant from the *Febaceae* family which grows in tropical region and the stem of the

plant has been used as traditional medicine since previous times.<sup>10</sup> Sappan wood contains active compounds in the form of flavonoids, which are homoisoflavonoid, brazilin, protosappanin and chalcone, saponin, terpenoid and tannin.<sup>11</sup> Brazilin acts as an antibacterial agent to inhibit the synthesis of amino acid and cellular proteins in bacteria. Thus, Brazilin has a high antibacterial efficacy toward *S. mutans*, a caries-triggering bacteria and *P. intermedia*, a negative gram bacteria that causes periodontal diseases.<sup>12,13</sup> In previous study, sappan wood was proven to be able to inhibit bacterial growth such as *S. typhi*, *K. pneumonia*, *E. coli*, *B. subtilis*, *P. aerogenosa* and *S. aureus*.<sup>14</sup> Sappan wood extract has an antibacterial effect against *E. faecalis*, *S. salivarius*, *S. sanguinis* and *A. viscosus*.<sup>15</sup> The aim of this study was to find the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of sappan wood ethanol extract (*Caesalpinia sappan L.*) toward the growth of *A. actinomycetemcomitans* and *P. gingivalis* which caused periodontal infections.

## MATERIALS AND METHODS

### Samples and Ethical clearance

The bark from sappan wood which has been crushed to a powder was collected by 1650 gram, then macerated twice with 96% ethanol, then inserted to a digital shaker with the speed of 50rpm for 24 hours and then filtered with a cloth filter to obtain filtrate. The filtrate was then evaporated using Rotary Vacuum Evaporator at the temperature of 40°C for 13 hours until a thick extract with 100% concentration was obtained.<sup>16</sup> This study was approved by the Health Research Ethical Clearance Committee of the Faculty of Dental Medicine, Universitas Airlangga, Indonesia, 206/HRECC.FODM/2018.

Preparation of *A. actinomycetemcomitans* and *P. gingivalis* culture

The colony of *A. actinomycetemcomitans* and *P. gingivalis* bacteria that was taken from the stock using sterile inoculation loop was inserted to the BHIB. After that, the bacterial culture in the BHIB medium was inserted to an anaerobic jar in an anaerobe state and incubated inside an incubator at the temperature of 37°C for 24 hours. Then, the culture's turbidity was observed to be equalized with the 0.5 McFarland standard ( $1.5 \times 10^8$  CFU/ml).

Determination of antibacterial activity

Eleven sterile reaction tubes were prepared and labeled with numbers 1-9, (+) for the positive control group and (-) for negative control group. The number 1 sterile tube was filled with 10ml of 100% sappan wood ethanol extract, and, in tube numbers 2-9, the positive control tubes and negative control tubes were filled with 5ml of BHIB medium. Then, 5ml of solution from tube 1 was added into tube 2, thus, half the concentration of sappan wood ethanol extract was obtained by mixing 5ml of BHIB and 5ml of 100% sappan wood ethanol extract. After that, 5ml of mixture was taken from tube number 2 and inserted in tube number 3 to obtain 25% concentration of ethanol extract. The procedure was repeated until tube number 9 so that a group of sappan wood ethanol extract with 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, and 0.39% was obtained. The positive control tubes contained BHIB medium and bacteria, and the negative

control tubes only contained BHIB medium; 0.1ml bacteria was then inserted into tube numbers 1-9 and the positive control tubes. All tubes were incubated at the temperature of 37°C for 24 hours to observe the turbidity after the procedure. Results from the dilution technique were then cultivated in the *Mueller Hinton* medium with streak method to obtain MIC and MBC number.<sup>17</sup> In order to obtain a more accurate result, 0.1 ml of bacteria from each tube, including positive and negative control, was subcultured with spreader method on a *Mueller Hinton* medium to count the number of bacterial colonies that had grown. The calculation of growing colony in each concentration was manually counted three times, each with a different observer.

Statistical analysis

The data of the bacterial colonies was analyzed statistically using SPSS version 17.0 (IBM, Armonk, New York, USA). We performed Anova to compare CFU between groups concentration and the post hoc analysis was performed using Tukey Honest Significant Difference Test (HSD).

## RESULTS AND DISCUSSION

According to the qualitative phytochemical analysis of the sappan wood ethanol extract, contents of flavonoid, alkaloid, saponin, and terpenoid active substances were discovered to have antibacterial potential (Table 1).

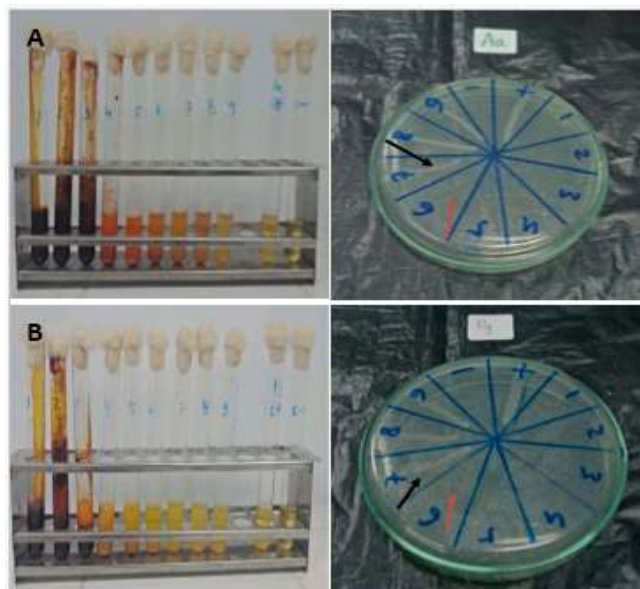
Table 1: Phytochemical Test Result on Sappan Wood Ethanol Extract

Active Substance	Phytochemical Test Result
Flavonoid	+
Tannin	+
Terpenoid	+
Saponin	+
Alkaloid	+

Notes: (+) indicates the existence of substances in the extract

Dilution method was carried out in order to find the antibacterial potential of sappan wood ethanol extract, (*Caesalpinia sappan*), hence, its Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC). This method was done by adding

sappan wood ethanol extract into BHIB, in which after, *A. actinomycetemcomitans* and *P. gingivalis* were inserted to be tested. The result of the dilution method continued with cultivation in each tube with streak method in a *Mueller Hinton* medium to obtain MIC and MBC (Figure 1).



**Figure 1:** Sappan Wood Ethanol Extract Antibacterial Potential toward *A. Actinomycetemcomitans* bacteria and *P. gingivalis*. Annotation: A. Observation of turbidity with dilution method and B. Observation of bacterial colony on the *Mueller Hinton* using streak method. Tube Number 1-9 consecutively with 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, and 0.39%. Tube with (+) mark was positive control and (-) mark was negative control. The area indicated by red arrow did not show growth of bacterial colony, area indicated by black arrow showed growth of bacterial colonies.

The cultivation of sappan wood ethanol extract in each concentration which had been administered with bacteria *A. actinomycetemcomitans* and *P. gingivalis* were conducted in *Mueller Hinton* medium with three replications. The result showed that, in the positive control, an average of 139 *A. actinomycetemcomitans* colonies was spotted, meanwhile, in the sappan wood ethanol extract with 100%, 50%, 25%, 12.5%, 6.25%, and 3.125% concentration and negative control, no bacterial colony growth. In 1.56%

concentration, an average of 12.667 colonies. Meanwhile, in each of the 0.78% and 0.39% were 31.67 and 42 colonies. The result from the calculation of *P. gingivalis* colony in positive control averaged at 138,667 colonies, at 100%, 50%, 25%, 12.5%, 6.25% and 3.125% concentration and negative control, there was no growth of *P. gingivalis* colony. The number of colonies on 1.56% concentration was 11,667 colonies and at 0.78% and 0.39% were 32,334 and 49,667 colonies, respectively (Table 2).

**Table 2:** Average of bacterial colony growth

Groups (Concentration)	n (repetition)	<i>A. actinomycetemcomitans</i> (CFU)	<i>P. gingivalis</i> (CFU)
100%	3	-	-
50%	3	-	-
25%	3	-	-
12.5%	3	-	-
6.25%	3	-	-
3.125%	3	-	-
1.56%	3	12,67 <sup>a</sup>	11,67 <sup>a</sup>
0.78%	3	31,67 <sup>b</sup>	32,33 <sup>b</sup>
0.39%	3	42,0 <sup>c</sup>	49,67 <sup>c</sup>
Positive Control	3	139,0 <sup>d</sup>	138,67 <sup>d</sup>
Negative Control	3	-	-

The different superscript letters in a column are significantly different ( $p < 0.05$ ).

## DISCUSSION

*A. actinomycetemcomitans* and *P. gingivalis* have a relationship to pathogenesis of the periodontal tissue. The ultrasonic scaling and laser therapy are efforts to decrease the severity of a disease, thus requiring other modes of therapy.<sup>18</sup> The result of sappan wood study revealed contents of active substances such as flavonoid, saponin,

alkaloid, tannin and terpenoid. Flavonoid can disrupt the formation of cell membrane and damage the permeability of bacteria's cell walls and inhibit the function of the cell membrane.<sup>19,20</sup>

Other study has also revealed that flavonoid can inhibit expression of inflammatory cytokine by lipopolysaccharide,<sup>21</sup> which are one of the main components

from negative gram bacteria virulence, such as *A. actinomycetemcomitans*.<sup>22</sup> *Brazilin* was included into the flavonoid group that had the potential as anti-inflammatory, antioxidant and antibacteria.<sup>23</sup> The tannin mechanism can inhibit DNA synthesis in the *A. actinomycetemcomitans* bacteria. Tannin can deactivate cellular adhesion of the *P. gingivalis* which, in turn, inhibits the enzyme that triggers protein transport, rendering it disrupted inside the inner membrane of the microbial cell.<sup>24</sup>

The effectivity of saponin as antibacterial agent worked by triggering leakage of protein and enzymes inside the cell and reducing the stability of the cell membrane. The active substance, terpenoid, can disrupt the formation process of cell walls and membranes; this caused the stability of the cell walls to be disrupted and killed the bacteria.<sup>25,26,27</sup> High quantities of alkaloids were found in various tissues of *C. sappan* twig. Alkaloids and their derivatives are used for analgesic, antispasmodic and antibacterial effects, anticancer activity and anti-inflammatory activity.<sup>28,29</sup>

The flavonoids, alkaloids, tannins, saponins, and terpenoids are active substances from sappan wood ethanol extract with each of its working mechanisms working in synergy to combat *A. actinomycetemcomitans* and *P. gingivalis* bacteria. Those mechanisms caused a decline in the physiological activity of the bacteria, which caused an inhibition toward the bacteria's growth, and, in turn, killed the bacteria.<sup>30</sup>

The result showed that 1.56 % concentration of sappan wood ethanol extract expressed an inhibitory effect toward the growth of *A. actinomycetemcomitans* bacteria at 90.887% toward the positive control group. This showed MIC value at the concentration of 1.56% , which according to the study conducted by Khan et al. (2016).<sup>31</sup> The MIC value of sappan wood ethanol extract toward *P. gingivalis* was obtained at 1.56% concentration at 91.587% inhibition rate toward the growth of *P. gingivalis* in positive control group.

Sappan wood ethanol extract was capable of killing *A. actinomycetemcomitans* at 3.125%, as it was observed that bacterial growth was halted at this concentration. The MBC value of sappan wood ethanol extract toward *P. gingivalis* was also at 3.125% because, at the appropriate concentration, there was no longer bacterial colony growth observed. The MBC was defined as the lowest concentration needed for an antimicrobial agent to kill 99.9% of bacteria. Increased concentration of sappan wood extract provides more active antimicrobial substances. Therefore, giving a higher concentration have a potential in inhibiting microbial growth. There were no differences observed in the sensitivity of the bacteria from sappan wood extract between *A. actinomycetemcomitans* and *P. gingivalis*.

## CONCLUSION

This study concluded that the growth of *A. actinomycetemcomitans* and *P. gingivalis* can be inhibited by sappan wood ethanol extract (*Caesalpinia sappan L.*) at MIC 1.56% and MBC 3.125% concentration.

## CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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