

eISSN 2549-6212  
pISSN 1979-0201

PADJADJARAN

**JOURNAL  
OF DENTISTRY**

JEMBER

PADJADJARAN

p-ISSN : 1979-0201 e-ISSN : 2549-6212

# JOURNAL OF DENTISTRY

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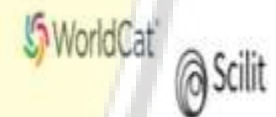
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## Inhibition of namnam (*Cynometra cauliflora* L.) leaves extract on the growth of *Porphyromonas gingivalis*

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### ABSTRACT

**Introduction:** *Porphyromonas gingivalis* growth should be prevented to minimise inflammation in periodontal tissues. Antibacterial herbs need to be considered because there are side effects caused by synthetic antibacterial drugs. Namnam (*Cynometra cauliflora* L.) leaves are known for having antibacterial effects. The purpose of this research was analysing the inhibition potential, and the highest inhibition concentration of namnam leaves extract on the growth of *Porphyromonas gingivalis*. **Methods:** 24 samples were divided into 6 groups. The positive control group was given 0.2% chlorhexidine, and the treatment group was given various concentrations (100%, 80%, 60%, 40%, and 20%) of namnam leaves extract. The disc which spilled by various concentrations of namnam leaves extracts and 0.2% chlorhexidine was placed on a medium which has been inoculated by *P. gingivalis*, then incubated at 37°C for 48 hours. The inhibition zone was measured using a vernier calliper. **Results:** The concentration of 100% had the highest average inhibition zone value, which was 11.43 mm. The content in namnam leaves extracts which serve as antibacterial were tannins, flavonoids, triterpenoids, saponins and quinones. **Conclusion:** Namnam leaves extract can inhibit the growth of *P. gingivalis*. 100% of namnam leaves extract has the highest antibacterial inhibition zone.

**Keywords:** Antibacterial, namnam leaves extract, periodontal disease, *Porphyromonas gingivalis*

p-ISSN 1979-0201; e-ISSN 2549-6212; Available from: <http://jurnal.unpad.ac.id/pjd/article/view/18540>

DOI: [10.24198/pjd.vol30no3.18540](https://doi.org/10.24198/pjd.vol30no3.18540)

Submission: Sep 02, 2018; Accepted: Jun 18, 2019; Published online: Jul 31, 2019

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### INTRODUCTION

Prevalence of dental and oral health problems are still relatively high (25.9%).<sup>1</sup> One of the most prevalent disease in the community is periodontal disease. Household Health Survey in 2012 results show that periodontal disease ranks second with a prevalence of ±70% of Indonesian population.<sup>1</sup>

Periodontal disease is the result of a complex interplay between subgingival biofilms and body immune cells that cause inflammation in the periodontal tissues in response to fighting bacteria. Periodontal disease includes gingivitis and periodontitis. Gingivitis is a periodontal disease that only affects the gingiva, while periodontitis is a periodontal disease that affects

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the deeper structures. This disease can cause bleeding gums, bad breath, tooth unsteadiness to lose teeth, disrupting the process of mastication.<sup>2</sup>

The cause of periodontal disease is multifactorial with bacteria in plaque as the primary cause. Among other periodontal pathogens, *P. gingivalis* is one of the critical pathogens in periodontal disease.<sup>2</sup> This bacterium is anaerobic gram-negative bacteria, which has several virulence factors such as enzymes, lipopolysaccharides, fimbria, and outer protein membranes, which can destroy the periodontal tissues directly or indirectly by inducing inflammation.<sup>3</sup> If the number of *P. gingivalis* colonies increases, periodontal tissue damage also increases. To prevent the rise of periodontal tissue damage, the growth of *P. gingivalis* must be inhibited.<sup>4</sup>

One way to inhibit the growth of microorganisms is by plaque control. Plaque control can be done mechanically by brushing teeth that depend on motivation. Individual motivation can decrease over time. Therefore, antiplaque chemicals are used to support mechanical plaque control.<sup>5</sup>

Plaque control is chemically carried out using a mouthwash containing antibacterial ingredients to clean areas that are not covered by toothbrushes.<sup>6</sup> The use of non-herbal mouthwash for a long time causes allergic reactions or abnormalities in the oral cavity. For example, the use of chlorhexidine for a long time can cause a burning sensation, changes in taste perception and the appearance of stains on the teeth.<sup>7</sup> Therefore, it is necessary to look for alternative ingredients from herbal ingredients to obtain minimal side effects. One of the natural ingredients currently developed as an antibacterial ingredient is namnam leaves (*Cynometra cauliflora* L.).

Namnam is the name of a fruiting tree species from the tribe of legumes (*Leguminosae* or *Fabaceae*) which often found in wet lowland areas.<sup>8</sup> This plant is one of the rare species of native Indonesian plants. Also, this plant grows in Southeast Asia and India.<sup>9</sup> This plant is often used by the fruit to be eaten fresh and leaves for diarrhoea medicine.<sup>10</sup> Namnam leaves contain chemical compounds such as flavonoids, terpenoids, tannins, antibacterial saponins.<sup>11,12</sup>

Research on namnam leaves is very limited until now there has been no study that tested antibacterial extract of namnam leaves (*Cynometra cauliflora* L.) against *P. gingivalis* bacteria. Based on descriptions above, the purpose of this research was analysing the inhibition potential, and the highest inhibition concentration of namnam leaves extract on the growth of *P. gingivalis*.

## METHODS

Type of this research is experimental laboratory. The research design is the post-test only control group design. This study used a total of 24 samples divided into 6 groups: K + (chlorhexidine gluconate 0.2%) and namnam leaves extract group concentrations of 100, 80, 60, 40 and 20%.

Research procedures include the preparation stage, inhibitory effect, inhibition zone measurement and data analysis. The preparation stage includes identification of *P. gingivalis* bacteria with gram staining, namnam leaves identification, tool sterilization using autoclave at 121°C for 30 minutes, namnam leaves extraction, making media and bacteria suspensions.

The extraction of namnam leaves is done by maceration technique. Namnam leaves were washed with clean water then dried for 10 days, then ovened at 40°C for 4 days. Dried leaves are mashed with a blender and sieved with an 80 mesh sieve.<sup>13</sup> Namnam leaves powder that has been sieved is taken 200 grams and put into a closed jar, then added 1000 ml of methanol. The maceration stage was carried out for 48 hours while stirring every eight hours using a stirring rod and then it was regenerated for 48 hours.<sup>13,14</sup> The filtrate was evaporated with a rotary evaporator at a temperature of 50°C to obtain concentrated extract with a concentration of 100%. Then the extract was diluted to a concentration of 80, 60, 40 and 20%. The dilution results were filtered using a syringe filter and inserted in the eppendorf tube.<sup>13</sup>

The next stage is the preparation of Brain Heart Infusion Broth (BHI-B) media and Brain Heart Infusion Agar (BHI-A). After making the media, followed by making a suspension of *P. gingivalis* bacteria. *P. gingivalis* used in this study

were obtained from the Microbiology Laboratory of Faculty of Dentistry University of Jember. That bacteria has been identified as pure *P. gingivalis* ATCC 33277. One ointment of *P. gingivalis* from pure strains was put in a vacuum tube containing 2 mL media BHI-B. Vacuum tubes were inserted into the desicator and incubated at 37°C for 24 hours. Then dilution was done by adding sterile distilled water and homogenized with mixing vortex, until the absorbance reached 0.5 Mc Farland measured using a densitometer.

The next procedure is the antibacterial effect test stage. A total of 100 µL of bacterial suspension was dripped on each culture medium. The suspension was flattened on the surface of the culture medium using sterile cotton swabs. On 24 pieces of disc paper, each of them was pressed with 13µL of namnam leaves extract with 5 concentrations and 0.2% Chlorhexidine using micropipette which was given a yellow tip and then waited for one minute to absorb. Paper discs are affixed to each surface of the culture media that has been inoculated with bacteria using sterile tweezers<sup>15</sup>. The petridish was inserted into the desicator and then incubated for 48 hours at 37°C in an upside down position to prevent water vapor from falling into the media so that it does not interfere with bacterial growth<sup>16</sup>.

The next procedure is the inhibition zone measurement stage using a digital shear term with 0.01 mm accuracy and recorded. The method of measuring the diameter of the inhibitory zone is by measuring the length diameter (a) plus the short diameter (b) then dividing it into 2<sup>17</sup> (Figure 1).

The results of the research data were Saphiro-Wilk and Levene statistical test using Statistical Product and Service Solution (SPSS) software. The resulting data is normally distributed and homogeneous, so that the One-Way ANOVA parametric statistical test can be carried out then

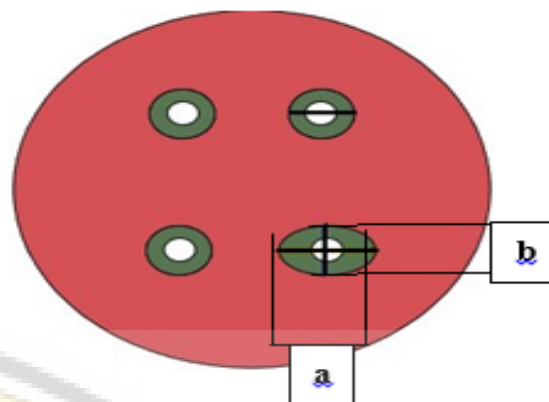


Figure 1. Inhibitory zone measurement

followed by the Tukey-HSD statistical test. All data tests used a 95% significance level ( $\alpha = 0.05$ ).

## RESULTS

The results of the research on the inhibitory power of namnam leaves extract (*Cynometra cauliflora* L.) on *P. gingivalis* growth obtained inhibitory zones around disc paper in all research groups. The results of the research data are presented in Table 1.

Table 1. The inhibitory zone diameter of namnam leaves extract on *P. gingivalis* growth

Groups	Inhibitory zone diameter ( $X \pm SD$ ) (mm)
K+	13.61±1.71
100%	11.43±0.66
80%	11.05±0.84
60%	10.62±0.64
40%	10.03±1.32
20%	8.59±1.03

Note: X: Mean; SD: Standard deviation; K+: Positive control group (Chlorhexidine gluconate 2%); \*significant difference

The results of the One Way Anova test showed significant differences between all study groups ( $p < 0.05$ ). Furthermore, the Tukey-HSD test was conducted to find out the differences between the 2 study groups (Table 2).

Table 2. The Tukey-HSD test of the inhibitory zone diameter of namnam leaves extract on *P. gingivalis* growth

Groups	P value					
	K+	100%	80%	60%	40%	20%
K+	-	0.105	0.041*	0.013*	0.003*	0.000*
100%	0.105	-	0.996	0.897	0.491	0.019*
80%	0.041*	0.996	-	0.993	0.777	0.052
60%	0.013*	0.897	0.993	-	0.972	0.146
40%	0.003*	0.491	0.777	0.972	-	0.459
20%	0.000*	0.019*	0.052	0.146	0.459	-

The results of the Tukey-HSD test in Table 2 showed a significant difference between the K + group with 80, 60, 40 and 20% group; and the 100% with 20% group ( $p < 0.05$ ). Whereas in the other groups there were no significant differences ( $p > 0.05$ ), which means that the diameter of the inhibitory zone between the two groups was almost the same.

## DISCUSSION

This study was aimed to determine the inhibitory power of namnam leaves extract (*Cynometra cauliflora* L) on the growth of *P. gingivalis*. Namnam leaves extract made using remaceration method. Based on previous research, remaceration extraction method yields the highest extract yield compared to other methods. This is because the contact time between the solvent and simplicia in the remaceration method is longer than other methods such as percolation, maceration and reperolation.<sup>18</sup>

The solvent used in the remaceration process is methanol. The use of methanol in this study because methanol is able to dissolve more phytochemical compounds than other solvents. Methanol has a polar group that is stronger than nonpolar groups, so it is able to extract more bioactive components that have higher polar properties such as saponins, quinones, flavonoids and tannins. This can be seen from the chemical structure of methanol containing hydroxyl (polar) and carbon (nonpolar) groups<sup>19</sup>.

The antibacterial power test method used in this study is disc diffusion method. The media to be tested is placed in a desiccator with a reversed condition. This can prevent droplets of water vapor from falling onto the medium that has been planted with bacteria, these droplets can affect the final result of incubation<sup>20</sup>.

The results showed that all namnam leaves extract groups had inhibitory power on *P. gingivalis* growth. The diameter of inhibition zone of namnam leaves extract concentrations of 100%, 80%, 60%, 40% and 20% were 11.43 mm, 11.05 mm, 10.62 mm, 10.03 mm and 8.59 mm respectively. This shows an increase in the diameter of the inhibitory zone as the concentration of extract increases, so that the namnam leaves extract 100% concentration has the greatest inhibitory ability than other

concentrations. This is presumably because the greater the concentration of an extract, the higher the content of antibacterial compounds so that the antibacterial ability is increasing. When compared with 0.2% chlorhexidine gluconat, namnam leaves extract 100% concentration has a smaller inhibitory zone diameter. Based on the Tukey-HSD test, it was found that  $p = 0.105$  showed that there were no significant differences between the groups so that it could be said that namnam leaves extract concentration of 100% and 0.2% chlorhexidine gluconate had almost the same ability in inhibiting the growth of *P. gingivalis*. This study proved that namnam leaves extract was able to inhibit the growth of *P. gingivalis*. This is consistent with previous research which states that namnam leaves extract has antibacterial activity<sup>21</sup>. The results of this research are in accordance with other research which states that namnam leaves extract has an antibacterial effect, but in *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* bacteria<sup>22</sup>. Other researcher also stated that the methanol extract of namnam leaves has antibacterial activity, namely in *Staphylococcus aureus* but not in *E. coli*<sup>13</sup>. The antibacterial properties found in namnam leaves extract are tannins, flavonoids, triterpenoids, saponins and quinones<sup>13, 23, 24</sup>. Each of these active substances has a different mechanism as an antibacterial.

Flavonoids work as antibacterials by inhibiting the formation of DNA and RNA which play a role in hydrogen bonds so that nucleic acid bases accumulate and the permeability of cell walls of bacteria, lysosomes and microsomes were damage<sup>25</sup>. Flavonoids can also form complex compounds with extracellular proteins, causing damage to bacterial cell membranes and followed by the release of intracellular compounds<sup>26</sup>. In addition, flavonoids are also able to inhibit cytochrome C reductase so that the use of oxygen in bacteria will be inhibited<sup>27</sup>.

Tannin compounds will damage bacterial cell membranes by changing permeability and disrupt the power of the cytoplasmic membrane protons that dissolve fat. In lower concentrations, tannins will activate enzyme systems in bacterial cells<sup>13</sup>. While saponins will bind to the cytoplasmic membrane so that the surface tension will decrease and cause the release of intracellular

compounds<sup>27</sup>. Other compounds, namely quinone are antibacterial by forming irreversible complexes with nucleophilic amino acids so that cell proteins cannot function normally<sup>28</sup>.

Antibacterial power strength can be divided into three, namely the diameter of the inhibitory zone of less than 10 mm is categorized as weak, the diameter of the inhibitory zone 10-20 mm is categorized as moderate and the diameter of the inhibitory zone of more than 20 mm is categorized as strong<sup>29</sup>. Therefore, the antibacterial power of namnam leaves extract on the growth of *P. gingivalis* can be divided into two categories, namely namnam leaves extract 20% concentration was included in the weak category, while namnam leaves extract concentration of 100%, 80%, 60%, 40% in the medium category. The difference in inhibitory zone size and antibacterial strength is influenced by several things, including the sensitivity level of the test organism, the speed of diffusion of antibacterial compounds and the concentration of antibacterial compounds<sup>30</sup>. This study is still limited to testing the antibacterial effects, especially *P.gingivalis* bacteria in namnam leaves extract. This article is still in the level of preliminary research, only the value of inhibition studied with no MIC value, so this research needs to be continued. Further research on toxicity and biocompatibility tests, as well as *in vivo* research on namnam leaves extract is also needed before this extract can be used in humans.

This study still requires further research to determine the Minimum Inhibitory Concentration (MIC) of namnam leaves extract against *P.gingivalis*. Further research needs to be done to test the other potential of namnam leaves extract for example as an antifungal, antioxidant, anticancer and antidiabetic.

## CONCLUSION

Namnam leaves extract can inhibit the growth of *P. gingivalis*. 100% of namnam leaves extract has the highest antibacterial inhibition zone.

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