

Antibacterial Potential of *Spondias pinnata* (L.f) kurz Leaf Ethanol Extract against *Streptococcus mutans* Bacterial Growth

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Wild mango (*Spondias pinnata* (L.f) kurz) or Cemcem or Kecemcem is one of the famous plants in Bali. It is widely used by Balinese as both food and traditional medicine. Several study has shown that *S. pinnata* leaf extract has antibacterial activity against several Gram-positive and Gram-negative bacteria. *S. mutans* is a Gram-positive bacteria that causes dental caries. In Indonesia, the prevalence of dental caries is 88.8% and most suffered by toddlers. The purpose of this study was to determine the effect of ethanol extract of *S. pinnata* leaves in inhibiting the growth of *S. mutans*. Inhibition zone test was carried out using the diffuse disc method with two extract concentrations of 60% and 80%, respectively. From the results, it was found that the inhibition zone of 60% concentration was 12.95 mm and 80% concentration was 15.77 mm. Both fall into the category of strong inhibition zones. Based on this, the ethanol extract of *S. pinnata* leaves can be used as a natural antibacterial agent.

Keywords: Antibacterial Activity; Ethanol Extract; *Spondias pinnata*; *Streptococcus mutans*.

Wild mango (*Spondias pinnata* (L.f) kurz) or Cemcem or Kecemcem is one of the famous plants in Bali. This plant has many benefit and it is widely used by Balinese as both food and traditional medicine for generations. "Loloh cemcem" is a traditional drink from Bali. It is made from cemcem (*S. pinnata*) leaves¹. Roots, bark, fruit, and leaves can be used in traditional medicine². *S. pinnata* is widely used by Balinese people as a medicine for fever and toothache. The pharmacological effects of *S. pinnata* are also used as food flavoring, antimicrobial, and anti-tuberculosis^{3,4}.

Research conducted by Wulansari and Armayanti (2008) on the use of *S. pinnata* leaves

extract concentrations of 20%, 40%, and 60% to inhibit the growth of *S. aureus*, *E. coli*, and *S. typhi* showed significant differences in the average inhibition power ($p < 0.05$) in all treatment, as well as the positive and negative controls⁵. Research conducted by Sudirga (2020) states that the *S. pinnata* plant is one of the traditional medicinal plants that has been used for generations by the people of the Trunyan Village. This plant has efficacy as a medicine for fever and toothache by using the leaves and sap from the *S. pinnata* which contains alkaloids, citric acid, and Ca-oxalate⁶.

Dental and oral health is often neglected by Indonesian people. The low awareness of maintaining dental and oral health is one of the

causes of dental and oral disease in Indonesian society. The results of the 2018 Basic Health Study (Risikesdas) showed that 57.6% of the Indonesian population experienced dental and oral problems and only about 10.2% received medical services. The largest proportion of dental problems in Indonesia is dental caries at 88.8% with the most suffers being children under five⁷.

Dental caries is an infection of the teeth caused by *Streptococcus mutans* bacteria which causes demineralization of the tissue, causing localized damage to the tissue. The main habitat of *S. mutans* is the mouth, pharynx, and intestines. Dental caries has several factors such as adhesion to the enamel surface, production of acidic metabolites, ability to build glycogen, and to synthesize extracellular polysaccharides⁸.

Seeing the phenomenon of dental caries caused by the influence of the proliferation of *S. mutans* contained in dental plaque, so the researcher wanted to examine the quality and antibacterial inhibition of the ethanol extract of *S. pinnata* leaves on the growth of bacteria that cause plaque formation. As well all known that dental plaque is the initial source of dental and oral disease.

MATERIAL AND METHODS

Preparation of *S. pinnata* Leaves

S. pinnata leaves are dried at 50°C for 15 hours, then ground with a blender machine to fine powder. The obtained *S. pinnata* leaf powder is then used in the extraction process.

S. pinnata Leaves Extraction Process

Extraction is performed by weighing 300 g of dried leaves, which are then dissolved in 96% ethanol up to 4500 ml. In addition, agitation and extraction were performed for 15 minutes using a microwave with a power of 450 watts. The obtained extract was filtered with Whatman Paper number 1. The obtained filtrate was concentrated in the rotary evaporator vacuum at 30°C.

In this study, the concentrations of the ethanol extract of *S. pinnata* leaves used were 60% and 80%, respectively. There are 4 treatments in this study. A negative control which is given treatment by giving ethanol solvent. A positive control that was treated with 2% chlorhexidine. Six times replication were conducted for high

accuracy. P1 was treated with ethanol extract of *S. pinnata* leaves with a concentration of 60%. P2 is a treatment with ethanol extract of *S. pinnata* leaves with a concentration of 80%.

Culture of *S. mutans*

Bacterial strains used were *S. mutans* ATCC. *S. mutans* were cultured into BHI-A with vitamin K. The agar media was made by 10 µl vitamin K, 50 µl hemin solution, BHI-A 37 g in 100 ml sterile distilled water and 500 µl yeast extract. One bacteria used from the ATCC bacterial stock and was inoculated, then incubated at 37°C for 24 h.

Preparing the *S. mutans* bacteria suspension

S. mutans suspension was made by incorporating one colony of *S. mutans* from BHI-A into liquid media with total volume of 10 ml containing 0.37 g BHI-B, 5 µl hemin, 1 µl vitamin K, and 50 µl yeast extract. Then the suspension was incubated for 24 h, and the concentration was measured to obtain turbidity equivalent to 1.5×10^6 CFU/ml.

Inhibition test of *S. mutans*

For antibacterial activity, the disc diffusion method was used. The *S. mutans* suspension was swabbed on the entire MH agar surface. The paper discs containing different concentrations of *S. pinnata* ethanol extract 60% and 80% respectively were placed on the agar surface. Then incubated at 37°C for 24 h.

The area without visible bacterial growth or clear zone around each disc was observed. The diameter of the clear zone was measured using a calliper.

Statistical analysis

The data obtained were analyzed for diversity with the One Way Anova test.

RESULTS AND DISCUSSION

The activity of *S. pinnata* leaves ethanol extract in inhibiting the growth of *S. mutans*, the One Way Anova test was used. The significance analysis are presented in Table 1.

Table 2 shows that the average inhibition of the control positive against *S. mutans* was 20.38 ± 0.12 . The inhibitory power of P2 was 15.77 ± 0.13 and the P1 was 12.95 ± 0.17 . Based on the results of the analysis using the One Way Anova test, it was shown that there was a significant

difference between the three groups ($p < 0.05$).

Graph 1 shows that there is a significant difference in the average inhibition zone between the three treatment groups.

The antibacterial test results of the ethanol extract of *S. pinnata* leaves against *S. mutans* showed a significantly different mean of inhibition zone ($p < 0.05$) between the ethanol extract of *S. pinnata* leaves 60%, 80%, and positive control (using chlorhexidine 2%). The diameter of inhibitory zone showed the increase in the average according to the increase in concentration. The average diameter of the inhibitory zone at concentration of 60% was 12.95 mm and a concentration of 80% was 15.77 mm. Pan *et al* (2009) claimed that the category of inhibition with an inhibitory diameter of 0 to 3 mm was classified as weak, while an inhibitory diameter of 3 to 6 mm was classified in the medium category, and an inhibition diameter greater than 6 mm was classified in the string category⁹.

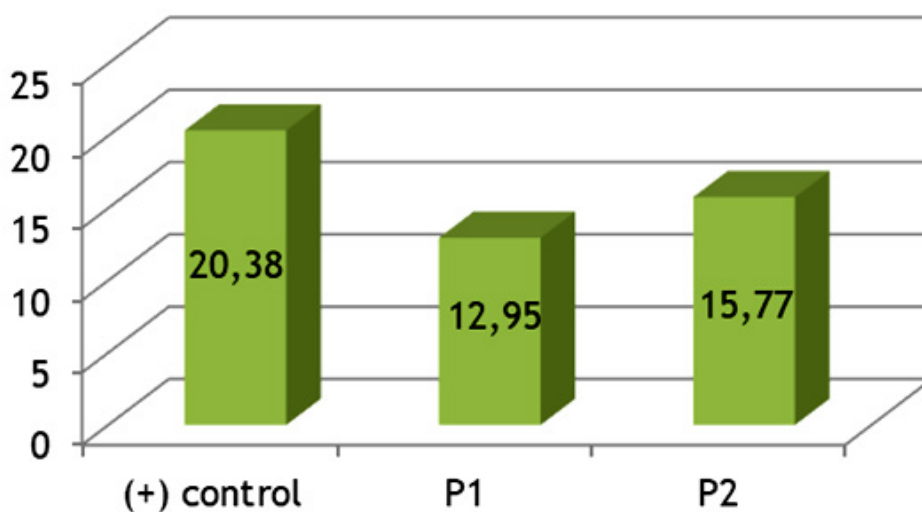
The results of this study are in line with research conducted by Wulansari *et al.*, (2018)

on the effectiveness of *S. pinnata* leaves extract to inhibit the growth of *S. aureus*, *E. coli*, and *S. typhi* bacteria. From the results of this study it was proven that there was a significant inhibition of bacterial growth⁵. Asnani *et al.* (2017) suggested that the ethanol extract of *S. pinnata* leaves contains steroids, flavonoids, tannins, and saponins is able to inhibit the growth of *S. aureus*, *K. pneumonia*, *M. morgani*, and *P. aeruginosa*¹⁰.

The content of phenolic compounds, flavonoids, saponins, and tannins in *S. pinnata* leaves extract will form a complex on the bacterial cell wall which causes inhibition and death of bacterial cells^{5,11-13}. Flavonoids are responsible for the observed antimicrobial activity. Flavonoids are a group of promising bioactive substances with low systemic toxicity. The leaves and stems are rich in flavonoids. The study by Adamczak *et al.* (2020) demonstrated moderate antibacterial properties of flavonoids against clinical strains of *E. coli* and *P. aeruginosa*¹⁴. Some studies identified that the antibacterial mechanism of flavonoids are inhibiting nucleic acid synthesis,

Table 1. The Differences in Bacterial Inhibition Zone between Groups after Treatment

Variable	Groups	n	Average±SD	p
<i>S. mutans</i>	Positive control	6	20.38±0.12	<0.001
	P1	6	12.95±0.17	
	P2	6	15.77±0.13	



Graph 1. The Differences in Inhibitory Zone of *S. pinnata* Leaves Ethanol Extract against *S. mutans*

inhibiting cytoplasmic membrane function by affecting biofilm formation, porins, permeability, and interaction with some key enzymes¹⁴⁻¹⁷.

S. mutans is a Gram-positive spherical bacterium that typically pairs or forms chains during its growth and is a normal flora of the oral cavity. *S. mutans* is able to synthesize large polysaccharides such as dextran from sucrose which is a sticky polysaccharide, and plays an important role in caries formation. The prevention and control of dental caries have been a major challenge for decades^{18,19}.

To date, prevention and treatment of dental caries is not limited to traditional methods used such as regular dental visits, brushing teeth with fluoride toothpaste, and low-sugar diets. Sogandi and Nilasari (2019) reported on the use of some natural ingredients as problem-controlling agents in the oral cavity and they found that noni fruits extract could inhibit the growth of *S. mutans* bacteria that cause dental caries²⁰. Suhendar *et al.* (2019) showed that the methanol extract of Kasturi mango contains alkaloids, flavonoids, phenolics, terpenoids, and saponins has an inhibitory activity against *S. mutans*²¹.

From the results of this study, it was found that the diameter of the inhibition zone of the ethanol extract of *S. pinnata* leaves was quite strong and could be used as an alternative treatment for dental caries.

CONCLUSION

This study demonstrated that the ethanol extract of *S. pinnata* leaves (cemcem) has the potential to prevent the formation of dental caries caused by *S. mutans* bacteria. *S. pinnata* leaves extract with 80% concentration provides an inhibition zone greater than 60% concentration. It can be used as a natural antibacterial agent.

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Conflict of Interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript. We certify that the submission is original work and is not under review at any other publication

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